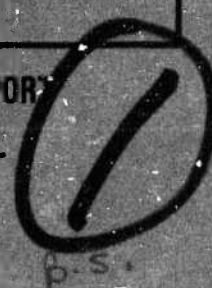


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TECHNICAL REPORT
76-55-AMEL



**A CALORIMETER DESIGN
FOR RAPIDLY ESTIMATING THE LEVEL
OF FOODBORNE MICROORGANISMS**

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December 1975

UNITED STATES ARMY
NATICK RESEARCH and DEVELOPMENT COMMAND
NATICK, MASSACHUSETTS 01760



Aero-Mechanical Engineering Laboratory

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Rapid means are needed for judging whether processed food prepared in a central food preparation facility is safe for consumption or not. The objective of this study is to define a design concept for suitable quality assurance instrumentation based on calorimetric measurement of the bacterial heat output. Heat production data were determined as a function of concentration for three aerobic mesophilic bacteria selected as representative of those to be found on contaminated food stuff. These data were determined at the temperature (37°C)		

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20. Abstract (cont'd)

and in a growth medium known to provide rapid growth for such bacteria. These data together with additional heat transfer studies and calculations were used to define the requirements for a multi-sample assay tool based on calorimetry for the determination of the level of microbial contamination of food. A design concept for this instrumentation is presented. Recommendations are made for further development of this assay technique.

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PREFACE

This study was carried out under the Materials Testing Technology Program administered by the Army Materials and Mechanics Research Center.

The authors wish to thank the following individuals who directly supported this effort: Mr. D. A. Mikelson who had developed the equipment originally and who helped us in reassembling the calorimeter apparatus and getting it into operation; Mr. Kim Boriskin of the NARADCOM Instrument Laboratory who assisted in defining the readout instrumentation requirements for the design concept; and Dr. Durwood Rowley, Dr. Hillel S. Levinson, Florence Feeherry and SP5 Hassan Srinivasa of the Microbiology Division of the Food Science Laboratory who advised us on the microbiological aspects of this study, provided the bacteria, and carried out the plate counts required to determine the growth curves.

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A CALORIMETER DESIGN FOR RAPIDLY ESTIMATING THE LEVEL OF FOODBORNE MICROORGANISMS

Introduction

The DOD food program has a requirement for a test apparatus and procedure which will provide a rapid means for judging whether processed food prepared in a central food preparation facility is safe for consumption or not. This Central Food Preparation – Satellite Dining Facility concept, under development by the Army, requires more storage and holding of precooked food than past practice; and these operations, if out of control, could produce a severe public health crisis and impair troop efficiency. Rapid microbiological quality measuring techniques are urgently needed to maintain control and preclude consumption of bad food. There are no such methods currently available or in use, either in the Army or the private sector.

One of the detection requirements is for the food to contain a typical plate count equal to or no greater than 10^5 aerobic mesophilic bacteria per gram of food at the time of sampling. A procedure is desired which will be suitable for operation by minimally trained personnel and will provide useful information within one to two hours. Present laboratory procedures require a minimum of 24 hours.

Detection techniques under investigation at the Natick Research and Development Command (NARADCOM) include radiometry and calorimetry (Rowley et al, 1974)¹. The microcalorimetric studies at NARADCOM reported by Rowley (reference 1) have demonstrated that the original bacterial content of *Staphylococcus aureus* in a sample can be determined on the basis of time required to reach a selected bacterial heat production rate. This work indicated that a calorimetric method may have the potential to meet the detection and time requirement. However, to fully develop or test the potential of this approach, the microbiologists at NARADCOM must examine a large number of samples which vary in food content, contamination level, and other preparative variables. The microcalorimeter which is currently available at NARADCOM and which was used in the previous work requires several hours to reach thermal equilibrium before meaningful heat production measurements can be made, and only one sample can be examined at a time, so that examination of many samples with this equipment is not practical. Since the long equilibration period requirement is inherent in the design of the present microcalorimeter, a new design specifically tailored to the needs of this investigative technique is required if the potential of this approach is to be fully assessed.

¹D. B. Rowley, J. J. Previte, R. E. Wells, Lampi, R. A. and D. A. Mikelson; "Radiometry and Microcalorimetry-Techniques for the Rapid Detection of Foodborne Microorganisms", Food Technology 28, p52, 1974.

The objective of this project is to define the design requirements and present a design concept for a rapid, multi-sample instrument based on the heat production of bacteria for use of the microbiologist. It is intended that this design should represent a first prototype of apparatus which, with further refinement as required, may ultimately be used for the routine assay of foods for aerobic mesophilic microorganisms in central food preparation kitchens.

The final design concept and procedure for use will not be unique but will represent the result of compromise and decisions made on several questions which are interrelated. The design effort requires that we attempt to define or establish:

- a. A sample preparation procedure including selection of a growth medium.
- b. The sample packaging procedure and configuration.
- c. The variations which must be anticipated in the heat production rates of bacteria as a function of time resulting from differences among bacteria in growth rates and heat evolved.
- d. A calorimetric system design which will allow for achievement of thermal equilibrium within the sample and between the sample and reference base in one hour or less, so that meaningful heat production rate data can be realized within 1 to 2 hours after sampling of the food.
- e. A system of calorimeter with associated readout instrumentation of sufficient sensitivity to provide for detection of the heat output of the bacteria by 1 to 2 hours after sampling if the food contains 10^5 or more bacteria per gram at the time of sampling.

Sample Preparation Procedures

The objective is to develop a sampling procedure which will minimize the dilution of the heat producing bacteria present in the food and to choose a growth medium and growth temperature which will maximize the growth rate and the heat production rate. However, sample preparation techniques and the selection of growth media providing maximum growth rates are not unique to calorimetric technique. Therefore, advantage was taken of recent studies (Rowley, 1975)² to develop a medium for use with the radiometric technique concurrently under study.

The composition of the growth medium adopted for this work is shown in Table I. This growth medium is considered suitable for rapid growth of the aerobic mesophilic bacteria which must be detected. In the heat production rate studies described later in this report, the samples in the vials used in the calorimeter were made by taking 12 ml of this medium at double strength, 10 ml of water, and 2 ml of a 0.1% peptone water with inoculum. In the one instance when anaerobic bacteria were studied, sufficient sodium thioglycolate was added to achieve a concentration of 0.1% in the vial in order to help maintain anaerobic conditions.

TABLE I

Growth Medium in Calorimeter Cell

Thiotone	1.5
Yeast Extract	0.5
Trypticase	0.5
Phytone	0.5
NaCl	0.5
K ₂ H PO ₄	0.25

It should be noted this medium was developed with emphasis only on achieving a rapid growth rate; it may not be the optimum composition for heat production and this factor should be explored. A greater energy release might be achieved with a different energy source which still might provide the same growth rates.

On the average, most rapid growth of the aerobic mesophilic bacteria of interest is achieved at 37°C; thus this temperature is adopted as the calorimeter operating temperature.

²Durwood Rowley, 1975. Personal Communication.

The sampling procedure objective is to achieve a desired growth rate without unnecessarily reducing the bacteria concentration level, which determines the heat production rate, when going from food to calorimeter cell. The procedure suggested Rowley (Reference 2) for sample preparation is the addition of the food sample by weight into the growth medium in a Waring blender. The use of a one-to-one or two-to-one ratio of growth medium to food is considered workable, and the final sample in the calorimeter cell will have the concentrations given in Table I plus the food sample pulp.

In summary, in adopting preparation procedures, advantage has been taken of previous studies to define the growth conditions which, on the average, provide rapid growth rates for the aerobic mesophilic bacteria of interest.

Sample Packaging

The original object in undertaking microcalorimetry at NARADCOM was to look for a method of testing the standard flexible food package for sterility. A calorimeter large enough to take the standard package was never developed, but the packaging materials and techniques were used to make a small pack that would fit in the Mound Calorimeter. The growth medium was sealed in the pack, and bacteria were injected using a hypodermic syringe, through a mass of silicone rubber sealant stuck on the side of the pack. This worked well, but several difficulties were foreseen in application to this study. The pack contained 13 ml of growth medium, and it was the practice to inoculate it with 0.4 ml of solution containing the bacteria. This practice made poor use of the measuring volume of the Mound Calorimeter, which is about 60 ml. The act of injecting 0.4 ml into 13 ml diluted the sample by a factor of 30; since the bacterial concentration increases exponentially, with a factor of 10 increase in an hour, this dilution will add 1 and 1/2 hours to the time needed to reach any pre-selected concentration. Furthermore, the bacteria level in the 13 ml volume will take 1/2 hour to grow by a factor of 3 to correspond to a heat output of a 40 ml volume sample. Thus conceivably the detection time could be reduced by over 2 hours by different packaging. However, with the inherent long equilibration time requirement of the Mound Calorimeter, full advantage could not be taken of this opportunity to reduce detection time.

Besides the above, the new series of tests were to be made by personnel not familiar with flexible packaging. For these reasons, it seemed best to try to find a sample bottle of the size and shape which would more effectively use the volume of the calorimeter sample chamber. Then we could increase the volume, make it easy to add the growth medium and inoculum and, even though food samples have not been used, make it possible to test food diluted only enough to add growth medium and enable it to be ground in a blender.

Other than dimensions, the most important consideration for a bottle is the cap. Any vapor leakage will tend to upset the calorimeter and make meaningful readings impossible. No screw cap bottle was found that was satisfactory. The bottle used was Virtis* No. 10-156TW-20, a thin wall type serum bottle. It was 28.6 mm in diameter, 61.5 mm high and held 25 ml. It was used with a fold-over rubber stopper that prevented any leakage (Virtis No. 10-151S). This stopper was the kind that could be pierced with a needle for inoculation. Although it wasn't used in this way, presumably it could be, for it should reseal as well as the silicone rubber used with the flex packs.

*The Virtis Company, Inc., Gardiner, N.Y. 12525.

Bacterial Heat Production Rates

Objective:

The design of suitable calorimetric instrumentation requires a knowledge of the growth and heat output characteristics of the bacteria to be detected — under the specific calorimetry conditions. With this knowledge, we can predict the heat production rate as a function of time for characteristic systems, so that the proper calorimeter design can be defined to meet the requirements of this investigative technique.

Prior Work:

Prior work at NARADCOM, as well as that reported in the literature, on heat output characteristics of bacteria as a function of their concentration has been very limited. Because of variations in growth media, growth temperature, bacterial concentrations, means of measurement, etc. from investigator to investigator, this work tends to raise more questions than it provides answers.

The prior work at NARADCOM (Reference 1) was with *Staphylococcus aureus*; only one run was reported which relates the heat production rate (HPR) as a function of time to the concentration as a function of time. Examination of figure 5 of their paper indicates that HPR is not proportional to bacterial concentration, but actually increases at a much slower rate than the concentration. Actually the HPR or ΔT (temperature gradient) expressed in μV on the figure is not directly proportional to a true heat production rate (P) except with a steady state system with $d(\Delta T)/dt = 0$ as shown below. The heat produced by the bacteria over a time interval dt is equal to the heat lost from the calorimeter in the interval dt due to temperature gradient ΔT plus the heat absorbed by the calorimeter over the interval dt .

This can be expressed as

$$P dt = k \Delta T dt + C d(\Delta T)$$

where P = heat production rate of bacteria

k = calorimeter constant

and C = heat capacity of calorimeter system

From this expression

$$P = k \Delta T + C \frac{d(\Delta T)}{dt}$$

and thus P at any time or concentration can be calculated from the ordinate and slope of the figure presented, but the ordinate alone represents a true HPR only if $d(\Delta T)/dt = 0$. Note that the derivation assumes a uniform calorimeter temperature which is not true, but also note that if the measured ΔT has a relatively constant relationship to the actual gradient, then changes in P should be meaningful. Derived data are presented in Table II. Exponential growth of the *S. aureus* appears to occur over the range of 10^4 to 10^8 cells/ml, and data taken from smooth curves indicate a heat production rate varying from 0.25 nW/cell at 10^4 cells/ml to 0.0013 nW/cell at 10^8 cells/ml. This represents a 200 fold decrease in P per cell with a 10^4 increase in cell concentration.

Calorimetric studies on *Staph. aureus* were also made by Bayne-Jones and Rhees (1929).³ Their data covered no more than two decades of concentration range, with exponential growth of the bacteria over the concentration range of 10^7 to 10^8 cells/ml. Their data were treated in the same manner and also indicate that the heat production rate does not increase in direct proportion to cell concentration. The growth rate was slower as shown in Table III and thus it is not surprising that P per cell at specific concentrations is lower than observed by Rowley *et al.* Good agreement is observed for the total amount of heat produced in going from a 10^7 /ml to 10^8 /ml cell concentration.

TABLE II

Data derived from Figure 5 of Rowley (Reference 1)

Cell Conc'n (ml)	P (μ W/ml)	P (nW/cell)
10^4	2.5	0.25
10^5	11.9	.12
10^6	37.7	.04
10^7	97	.01
10^8	127	.0013

³Bayne-Jones, S. and H. S. Rhees; "Bacterial Calorimetry, II, Relationship of Heat Production to Phases of Growth of Bacteria", J. of Bacteriology 17, p123, 1929

Table III

Comparisons of Prior Work

Bacterial Study	Heat Production over 10 ⁷ -10 ⁸ conc'n Interval (μW-hr/ml)	Time Period (hrs)	P at cell conc'n	
			10 ⁷ (nW/cell)	10 ⁸ (nW/cell)
<i>Staph. aureus</i>				
Rowley et al	121	1.1	.01	.0013
Bayne-Jones	114	1.9	.005	.0007
<i>Aerobacter arogenes</i>				
Stoward	175	2.3	.0058	.0009
<i>E. coli</i>				
Bayne-Jones	190	1.7		

Stoward (1962)⁴ studied *Aerobacter aerogenes* over the concentration range from 10^7 to much greater than 10^8 .⁵ bacteria per ml, finding an exponential growth rate of bacteria over the concentration range of 10^7 to 10^8 .⁵ He also remarked on the sharp decrease in heat output per cell with increasing concentration. Data from this paper are also represented in Table III. His results were criticized by Forest & Walker (1962)⁵ who claimed that the effect observed was due to time lags in the calorimeter, and who showed measurements of their own that did not vary with concentration. The results of Forest & Walker (reference 5) were on different bacteria and were apparently taken over a much more limited concentration range. Their criticisms were refuted by Stoward⁶ in subsequent comments, and based on the results of Rowley et al (reference 1) and Bayne-Jones (reference 3) as reviewed here, the effect of decreasing heat output per bacterium with increasing concentration does appear to be real.

⁴Stoward, P. J., Nature 194, p977, 1962.

⁵Forest, W. W. and D. J. Walker, Nature 196, p990, 1962.

⁶Stoward, P. J., Nature 196, p991, 1962.

Baynes-Jones and Rhees (reference 3) also studied *E. coli*, and a result is shown in Table III for this bacterium. The data is presented in the paper were for a corrected rather than observed temperature, and thus heat production rates were not calculated for Table III.

The prior work implies a decrease in heat output per bacterium with increasing concentration; below some concentration one might expect a constant value, but there is no experimental knowledge. The heat production does appear to vary with growth rate in a predictable manner, but there is no knowledge of how heat production rates may vary with concentration for the different bacteria of interest to this study under a given set of growth conditions. Unfortunately, recent bacterial calorimetry studies (see for example Russell et al)⁷ have been directed towards using the shapes of the heat production vs time curves for identification of bacteria and have not included data on bacterial concentrations as a function of time.

Data Needs:

The prior work does not define the rates of heat production to be expected for bacteria of interest under the specific growth conditions selected and at the concentration ranges of most interest.

Unfortunately, the bacterial populations to be found on food are not unique; the populations can be expected to differ from sample to sample, in number and ratios of specific bacteria. Therefore, any attempt to obtain quantitative information for design purposes from contaminated food was not considered a fruitful approach at this time. On the other hand, it is not possible because of time and funding limitations to study all aerobic mesophilic bacteria in pure cultures. Consequently three bacteria considered representative (reference 2) of those to be detected were selected for study, in order to obtain a first approximation of the variations to be found in bacterial growth and heat output characteristics, under the selected calorimetry conditions of temperature and sample nutrient concentrations.

The aerobic mesophilic bacteria selected for study were *Staphylococcus aureus*, *Salmonella typhimurium*, and *Bacillus cereus*. In addition, *Clostridium perfringens* was examined to obtain an indication of the performance of an anerobe.

⁷ Russell, W. J., J. F. Zettler, G. C. Blanchard, E. A. Boling, "Bacterial Identification by Microcalorimetry". Chapter 7 in *New Approaches to the Identification of Microorganisms* edited by Carl-Goran Hedén and Tibor Illén, John Wiley & Sons, 1975.

Experimental Procedures:

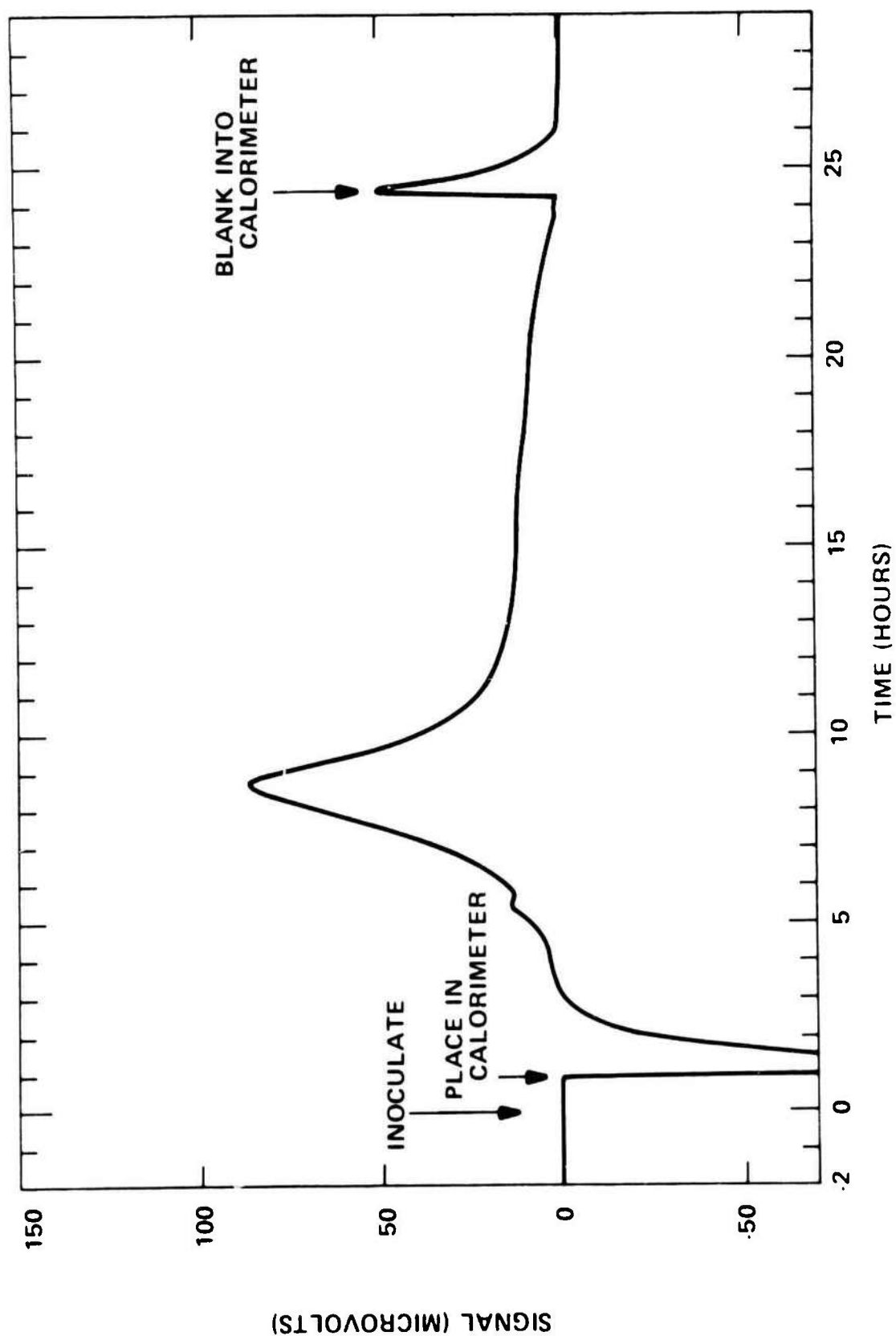
For each test, twelve identical samples in bottles were prepared as described earlier in the section on sample preparation procedure. One was immediately used to determine the initial number of bacteria/ml. Another was taken for the heat studies and placed in a preheater at 37°C to bring it close to the calorimeter temperature; after about an hour this sample was transferred to the calorimeter and the rate of heat production was measured as a function of time. The others were placed in an auxiliary bath maintained at 37°C; one was removed periodically from this set to determine the bacterial cell count as a function of time. The presumption is that the bottle in the calorimeter has the same concentration of cells at the time of sampling as found in the sample bottle removed for examination. All counts were made by a standard plate count method. Thus for each experimental run, two curves were obtained: one providing a signal related to the rate of heat production as a function of time, and the other relating to bacterial concentration in the number of cells/ml as a function of time.

The heat measurements at NARADCOM were carried out in a twin calorimeter (borrowed from The Mound Laboratories and described previously in Reference 1 using a bridge circuit to detect temperature differences. The calorimeter constant was determined by applying a measured electrical input to the sample chamber with a dummy sample present and obtaining the resulting equilibrium temperature difference between the calorimeters. This calibration gave a calorimeter constant of 18.5 watts per volt of temperature difference. In this system 2 μ V are detectable and are equivalent to about 10⁻⁴°C temperature difference; thus a 37 μ W steady state heat production rate will result in a measurable output of 2 μ V corresponding to about 10⁻⁴°C temperature difference between the calorimeters.

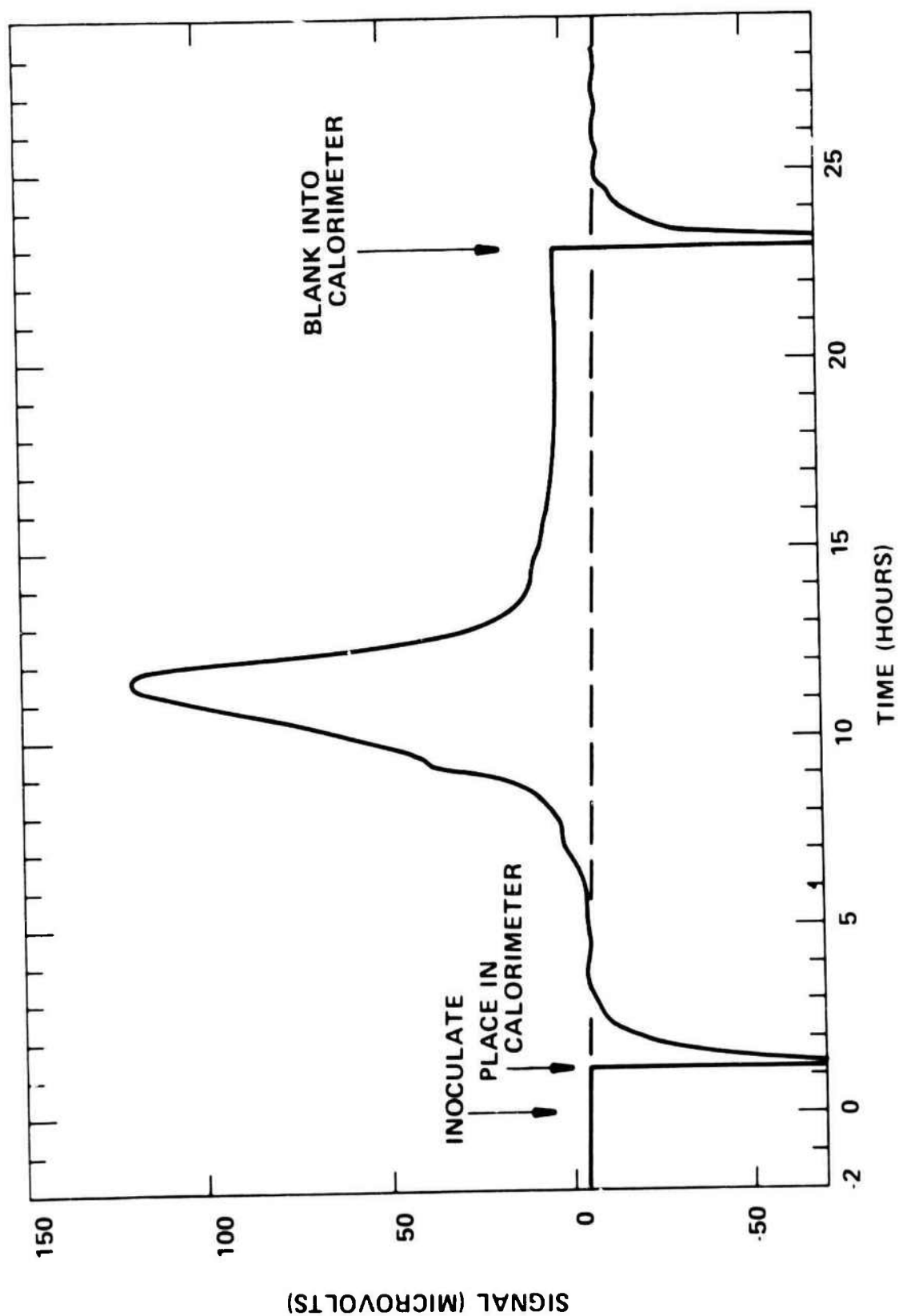
Experimental Data:

Two runs each were made on *Staphylococcus aureus* and *Salmonella typhimurium* and one each on *Bacillus cereus* and *Clostridium perfringens*. The results of the calorimetry are shown in Figures 1 to 6, and the results of the plate counts on the samples examined as a function of time are presented in Figures 7 and 8.

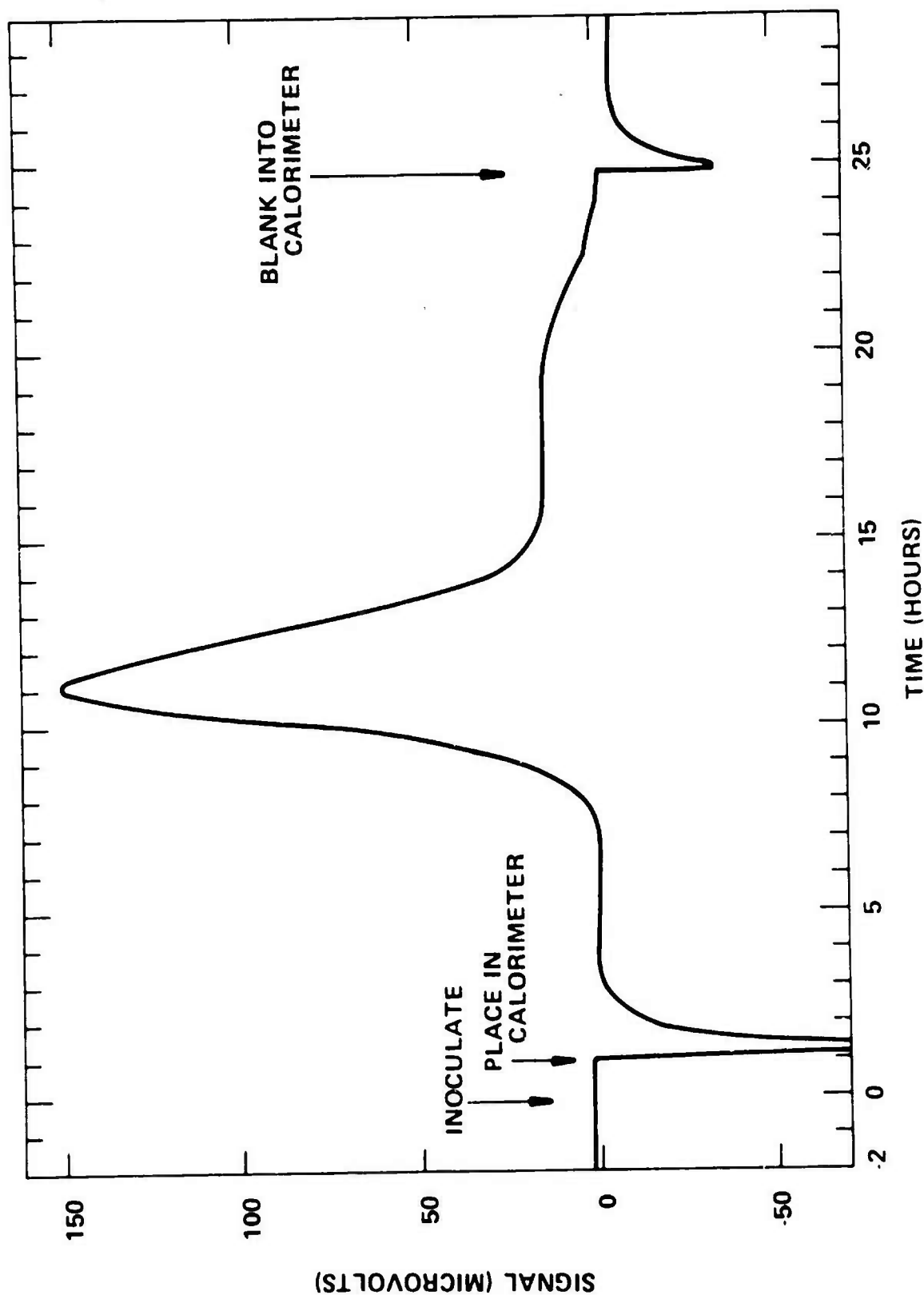
In Figure 6, the heat production curve for the anaerobe *Clostridium perfringens*, there was an early rise above the zero line. This behavior was questioned, so a sample bottle containing the same growth medium, but using sterile water in place of the bacteria was run. The same early rise was observed. The growth medium for this anerobe was the same as for the aerobic bacteria except that sodium thioglycollate was added to help maintain anaerobic conditions. It is apparently the reactions associated with this component that produce the early heat. The signal observed in the blank test was subtracted from the original data to provide the curve corrected for this nonbacterial heat contribution.



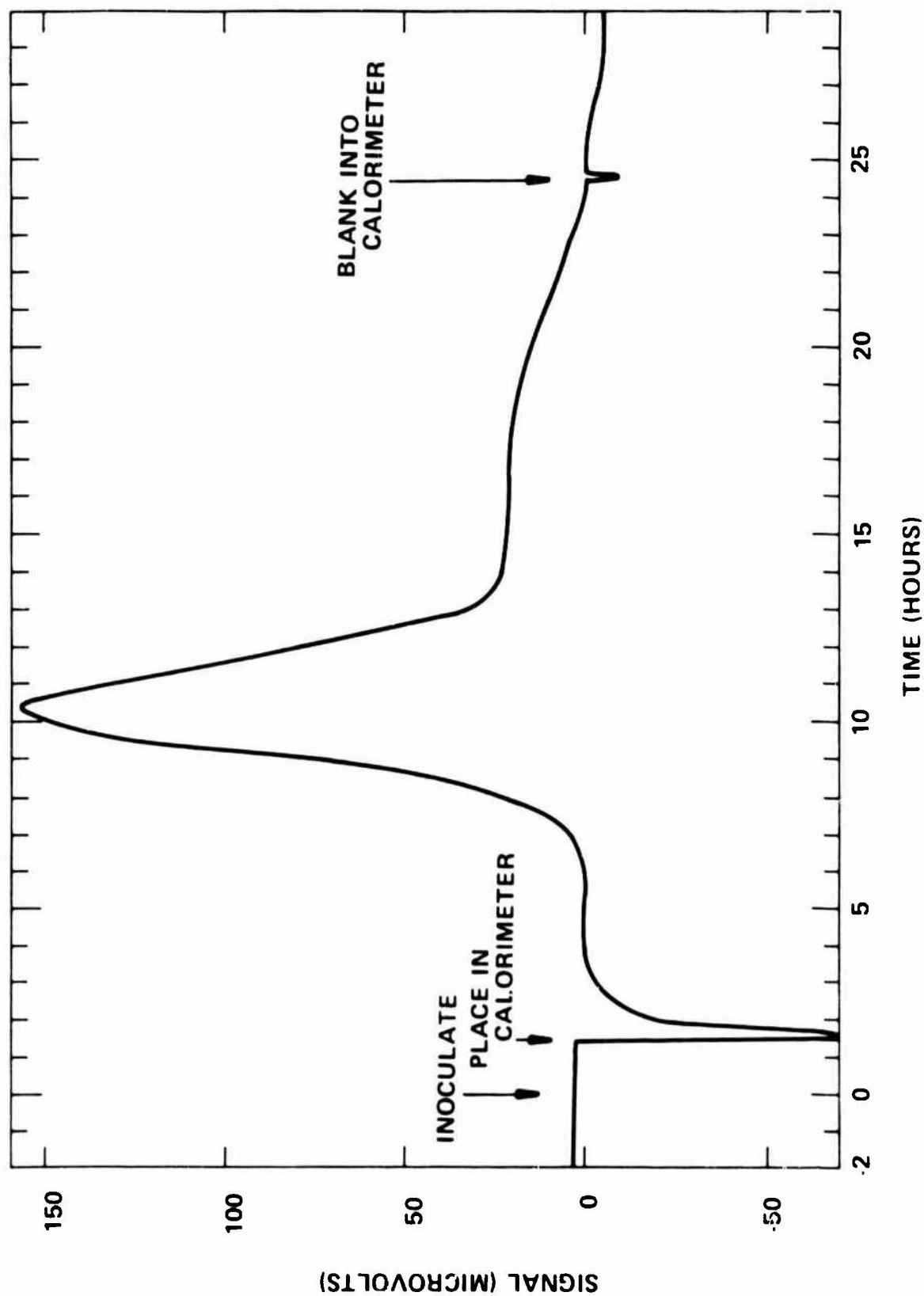
**FIG. 1: CALORIMETRIC RESULTS FOR STAPHYLOCOCCUS AUREUS
(ORIGINAL COUNT OF 608 CELLS/ML.)**



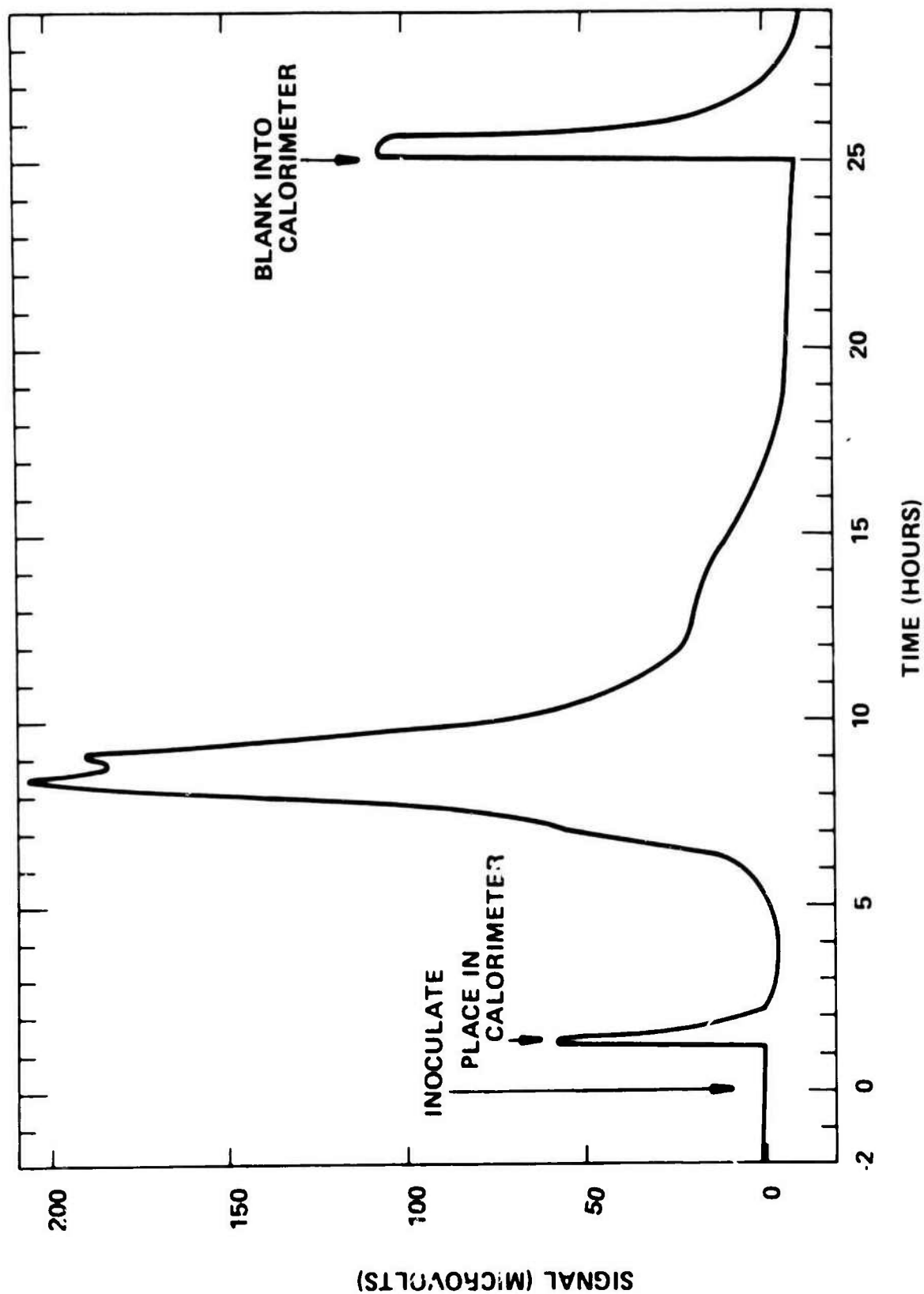
**FIG. 2: CALORIMETRIC RESULTS FOR STAPHYLOCOCCUS AUREUS
(ORIGINAL COUNT OF 17 CELLS/ML.)**



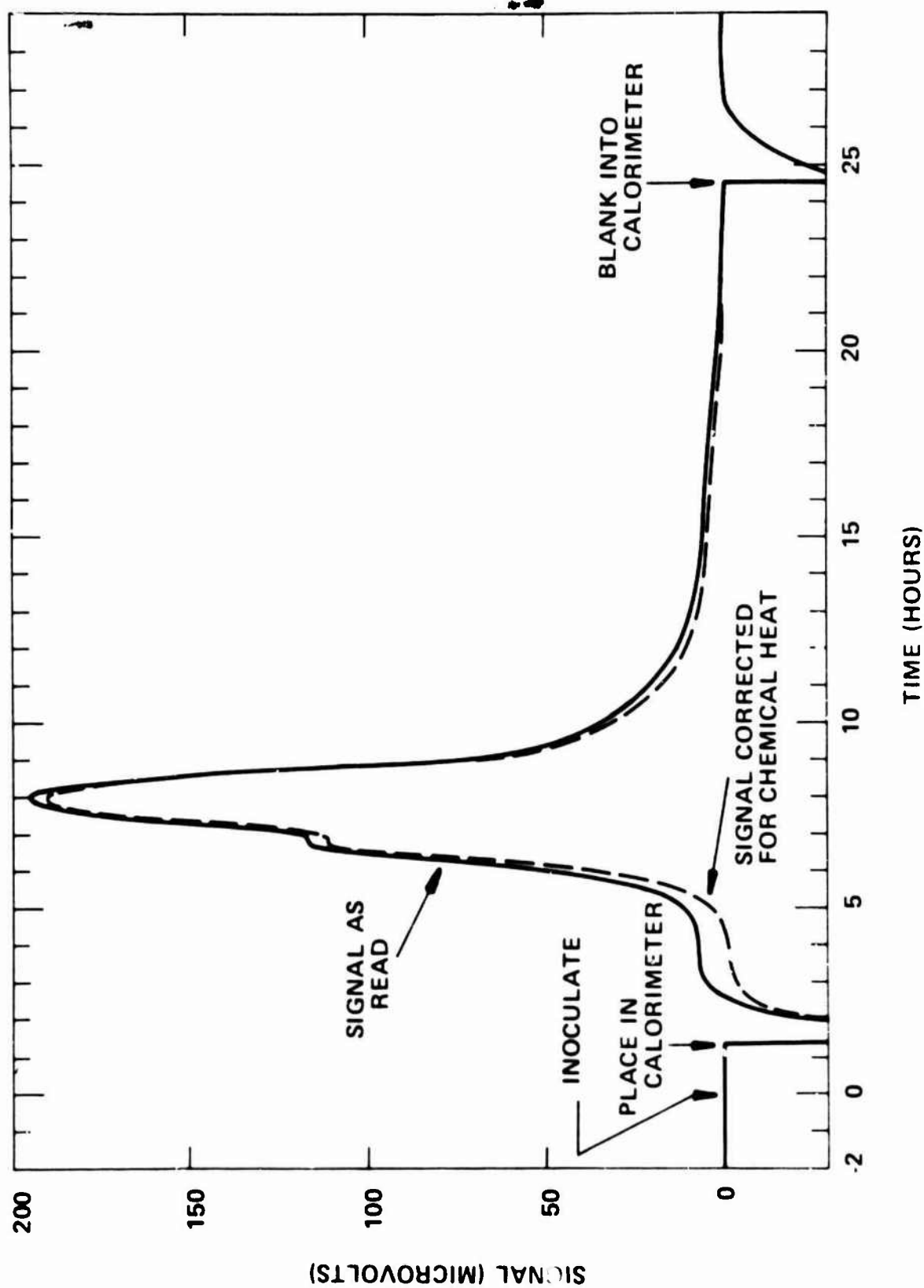
**FIG. 3: CALORIMETRIC RESULTS FOR SALMONELLA TYPHIMURIUM
(ORIGINAL COUNT OF 4 CELLS/ML.)**



**FIG. 4: CALORIMETRIC RESULTS FOR SALMONELLA TYPHIMURIUM
(ORIGINAL COUNT OF 22 CELLS/ML.)**



**FIG.5: CALORIMETRIC RESULTS FOR *BACILLUS CEREUS*
(ORIGINAL COUNT OF 24 CELLS/ML.)**



**FIG. 6: CALORIMETRIC RESULTS FOR CLOSTRIDIUM PERFRINGENS
(ORIGINAL COUNT OF 34 CELLS/ML.)**

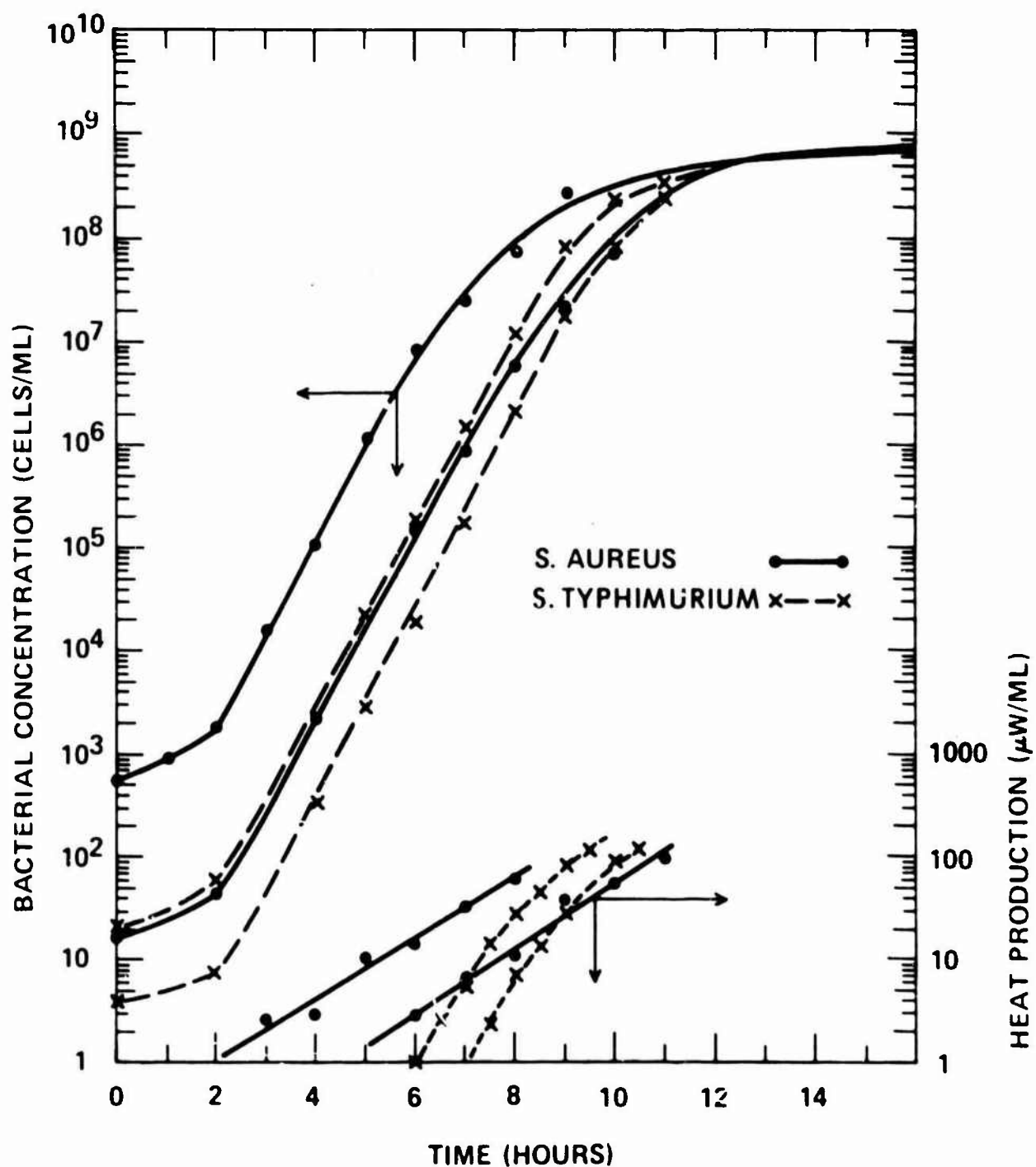


FIG. 7: BACTERIAL GROWTH AND HEAT PRODUCTION DATA FOR STAPHYLOCOCCUS AUREUS AND SALMONELLA TYPHIMURIUM.

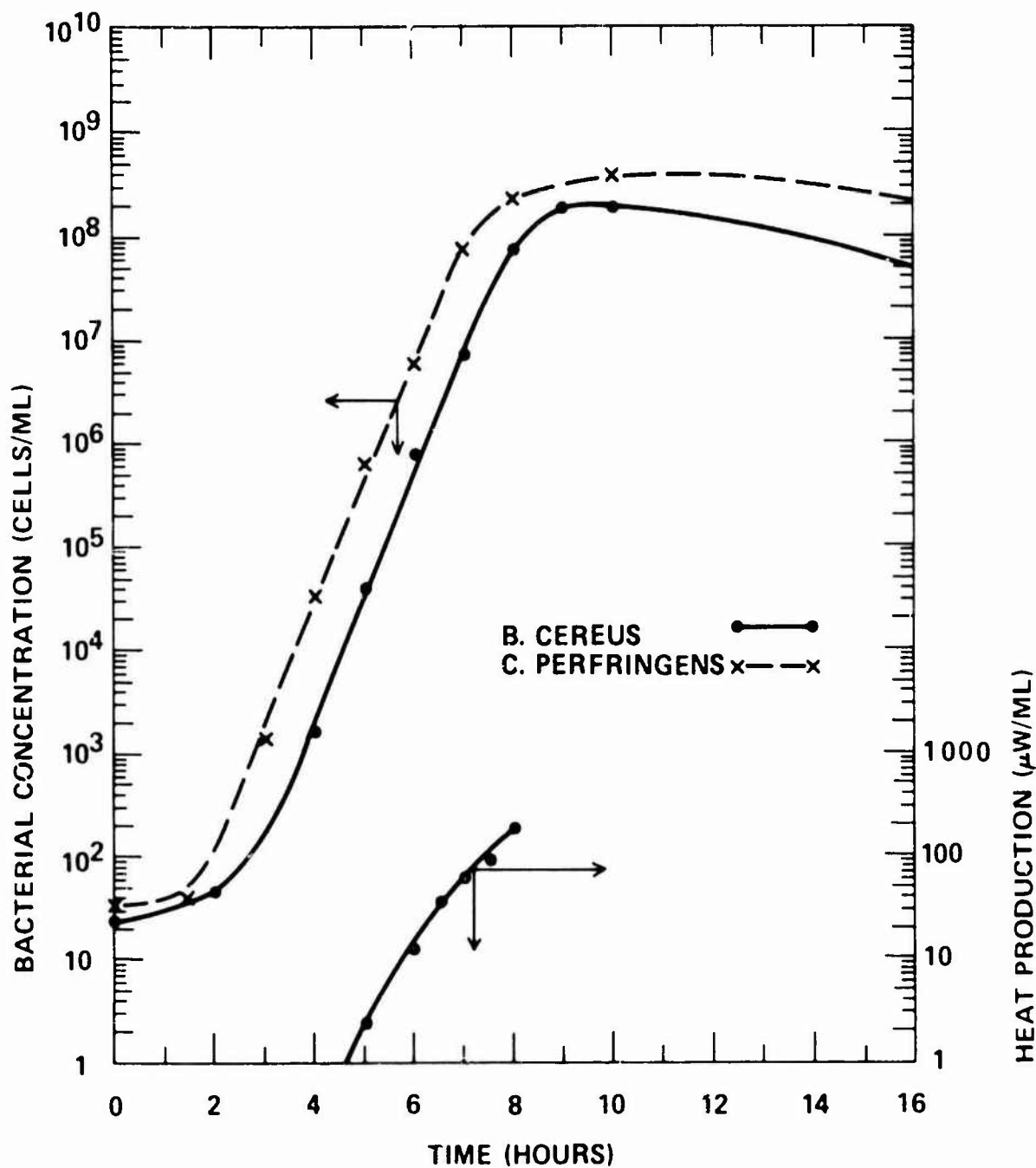


FIG. 8: BACTERIAL GROWTH AND HEAT PRODUCTION DATA FOR BACILLUS CEREUS AND GROWTH DATA FOR CLOSTRIDIUM PERFRINGENS.

Discussion of Data:

The raw calorimetric data of Figures 1-5 were used to provide the true heat production value (as discussed previously and described in detail in Appendix A), and these data for the aerobic bacteria are plotted on Figure 9 against the corresponding bacterial concentration. These same data are plotted on Figures 7 and 8 as a function of time. These measurements confirm that heat production per ml does not increase directly with concentration except possibly at the lowest concentration range observed for *Salmonella*. The data obtained from duplicate runs on *Staph.* and *Salmonella* are in good agreement. The data for the three aerobes indicate, in our opinion, a very close agreement between heat output and cell concentration (as shown on Figure 9) for the various bacteria in the given growth medium. If heat production rate alone was taken as a measure of cell concentration, and assuming that other aerobic mesophilic bacteria fall within the same band, then a prediction of cell concentration could be made which would be correct to within one order of magnitude. Note that the results obtained by Rowley et al (Reference 1) on *Staph.* and summarized in Table II are somewhat higher than those obtained in this study; we attribute this difference to the use of a different growth medium, and the results substantiate our belief that the growth medium composition should be studied to optimize heat production while still providing a universal growth medium for the aerobic, mesophilic bacteria.

The growth curves are normalized to a starting concentration of 1 in Figure 10. Again we see good agreement between duplicate runs even though these were originally measured over somewhat different concentration ranges. We would expect some decrease in the lag period with increasing initial concentration⁵; this effect may be present in the data observed, but it is too small to be significant at this time in our study. The normalized growth curves of Figure 10 and the heat production data from Figure 9 were used to derive a heat production vs time curve assuming a starting concentration of 5×10^4 cells per ml. Such a starting concentration would result from food samples containing 10^5 bacteria per gram and a one-to-one ratio of food to growth medium used in the preparation procedure, as discussed previously. Note that these curves (also shown in Figure 10) demonstrate that for the *Salmonella*, which is the slowest to produce heat, a 3 hour growth period would be required before meaningful heat output measurements (resulting from bacterial heat outputs of about $5 \mu\text{W/ml}$) could be obtained with a calorimeter design as sensitive as the Mound Calorimeter used in these studies. For other bacteria such as *Staph. aureus* and *B. cereus*, significant outputs could be detected at about 2 hours time. Assuming that all aerobic mesophilic bacteria heat production falls within the band observed for those bacteria selected for study, then one could say that heat outputs detectable between 2 to 3 hours or less would indicate food containing 10^5 or greater bacterial count per gram. This does fall within the desired one to four hour detection time as discussed in the Introduction. There is good reason to believe that this time requirement could be still further reduced by proper selection of the growth medium and use of a more sensitive calorimeter.

⁵ Lamanna, Carl and M. Frank Mallette, "Basic Bacteriology", Williams and Wilkins Co., Baltimore, 1965.

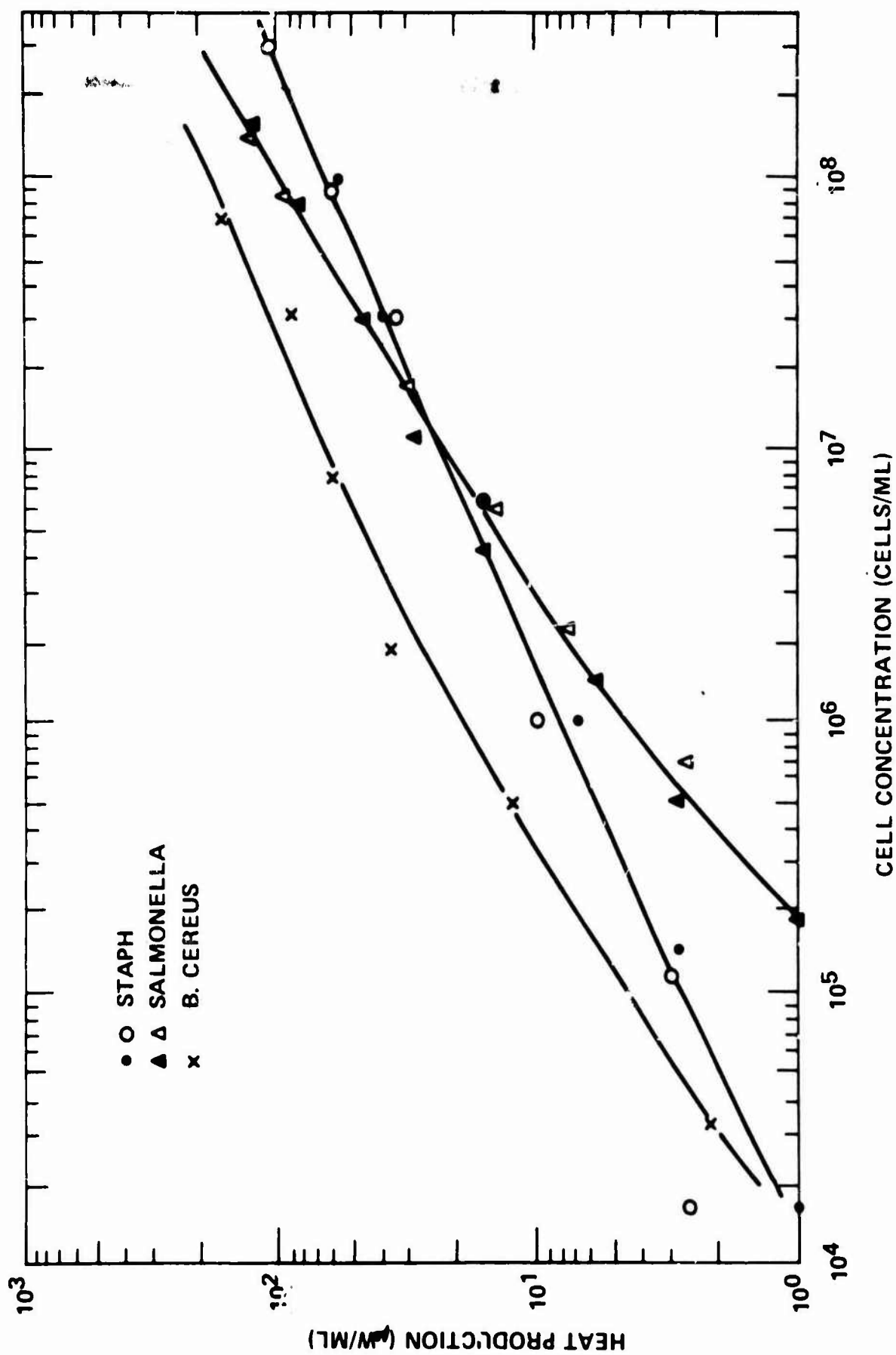


FIG. 9 : BACTERIAL HEAT PRODUCTION VS CELL CONCENTRATION.

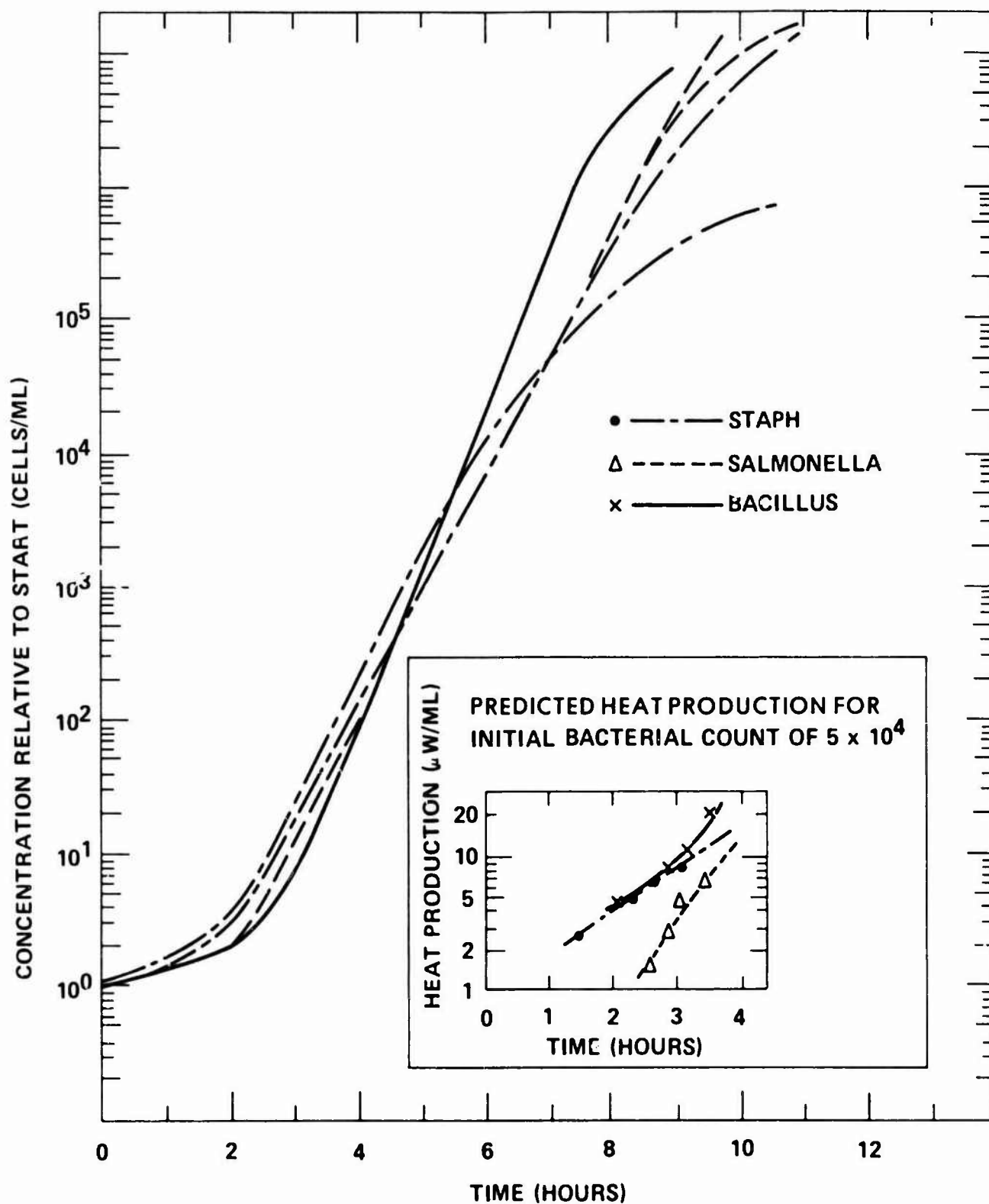


FIG. 10 : NORMALIZED GROWTH CURVES AND HEAT PRODUCTION PREDICTION.

In summary, the results obtained on growth rates and heat production characteristics for the three representative aerobic mesophilic bacteria vary within a relatively small range for the selected growth conditions. This fact supports the potential feasibility of a rapid quality control technique based on calorimetry. The results also provide a basis for detailed design of a calorimeter to meet the multi-sample testing needs of the microbiologist. Such a tool is required to further define and develop the calorimetric technique for rapid detection of food borne microorganisms.

Design Concept Development

Requirements:

The object of this calorimetric system is to detect bacterial growth in the sample from the metabolic heat evolved; the measured level of heat production as a function of time from sample preparation will indicate the initial bacterial concentration in the food. A rapid recognition of contamination levels exceeding allowable limits is required to properly control the food preparation operations, and thus a system that senses low levels of heat output and permits meaningful data collection in a short period of time is required. The heat production will continue for many hours with the heat production level increasing with concentration; there is no need for measurement of absolute quantities evolved or for measurement of the total heat evolved, but rather the need is to measure a quantity that relates to the rate of heat production and that is sensitive to variations in the rate.

The heat production measurements discussed in the last section demonstrate that a calorimeter system sensitivity in $\mu\text{W}/\mu\text{V}$ at least equal to that of the Mound Calorimeter used in these studies is required. Furthermore the results indicate that for such a system sensitivity, meaningful heat production rates must be measurable within two hours from the time of inserting the sample; this requirement means that the new design must permit the sample and the calorimeter system to achieve thermal equilibrium in a time period on the order of one to two hours rather than the four to five hours required with the Mound Calorimeter.

The final design should handle at least 10-12 samples at a time and be capable of use in a routine fashion with a minimum of training. Certainly cost of the total system may be a factor in selection among alternative assay techniques, and the design must reflect this consideration.

The Equilibration Problem:

The requirements for a high sensitivity together with rapid equilibration of the sample and calorimeter with the reference/surroundings when the sample is first introduced are not usually met in a single calorimeter design. The thermal isolation of the calorimeter from its surroundings, necessary to achieve high sensitivity will usually impose a requirement for long equilibration times prior to initiating measurements.

One approach to meet these requirements is to add heat to the sample in the isolated calorimeter by means of a built-in heater in order to bring the sample rapidly to the temperature of the reference/surroundings. Rapid equilibration to within the temperature differences desired requires careful control of the heat input. This approach was used in a design patented by the Instrumentation Laboratory, Inc. of Lexington, Mass.⁹ This

⁹Patent Specification 1341944 published 28 Dec 1973 by the Patent Office, London and titled *Improvements in or Relating to Apparatus for Calorimetry*.

design was apparently used subsequently in the work reported by Russell (reference 7). This system used an integral computer system for data acquisition, processing, and display, as well as for complete instrument control and monitoring. Operational characteristics of the final instrument development have not been published (beyond the Patent Disclosure); the instrument is no longer operational, and personnel conversant with the development were no longer available at Instrumentation Laboratory, Inc. The patent indicates an equilibration time on the order of 2½ hours, which is longer than we desire. We have no reason to believe that equilibration could not be achieved in shorter times by this approach, but the computer and associated control required by this design is very expensive. This design would make the cost of the desired quality control instrumentation several times more expensive than we believe can be achieved by an alternate approach. Thus we have chosen not to follow this design avenue.

An alternative approach is to seek a system design which will provide high heat-transfer rates from the calorimeter sample chamber to the surroundings, in order to achieve rapid equilibration in the system when the sample is first introduced, and which will also provide low heat-transfer rates during the heat measurement period in order to achieve a high sensitivity to low heat-production levels; high rates of heat transfer within the calorimeter sample chamber itself are necessary to fulfill both requirements. In principal, this need for controlling heat flow between the calorimeter sample chamber and its surroundings can be accomplished by thermally "shorting" the calorimeter sample chamber to its surroundings with a high conductivity shunt during equilibration followed by removal of the shunt resulting in isolation during the measurement period. In practice, one way to achieve such a thermal shunt is by alternate use of a highly conductive gas such as hydrogen or helium and a nonconducting vacuum between the calorimeter sample chamber and the surroundings. If helium is selected for reasons of safety, it appears possible to vary the conductivity 1000 fold between pressures of 1.01×10^5 Pa (1 atm.) and 1.3×10^{-2} Pa when using a 5-mm gap. The 5-mm gap appears to be reasonable for construction, and the lower pressure limit cited can be readily achieved with a good rotary oil pump. Other alternatives include the use of liquid such as mercury in place of helium or the use of mechanically actuated metal shunts.

The equilibration problem together with numerous conductance calculations are presented in Appendices B and C for the calorimeter configuration shown in Figure 11. The design considerations relating to equilibration within the calorimeter sample chamber and between the calorimeter sample chamber and the surroundings will be summarized in the next few paragraphs.

The Mound Calorimeter measures temperature difference between two twin calorimeters using a bridge circuit. The configuration shown in Figure 11 uses thermocouples between a sample chamber and a thermal reference block. These elements are within a Dewar vessel which can be filled with helium or evacuated to vary the rate of heat flow between the sample chamber as well as thermal reference and the constant temperature bath. This choice of configuration has several advantages:

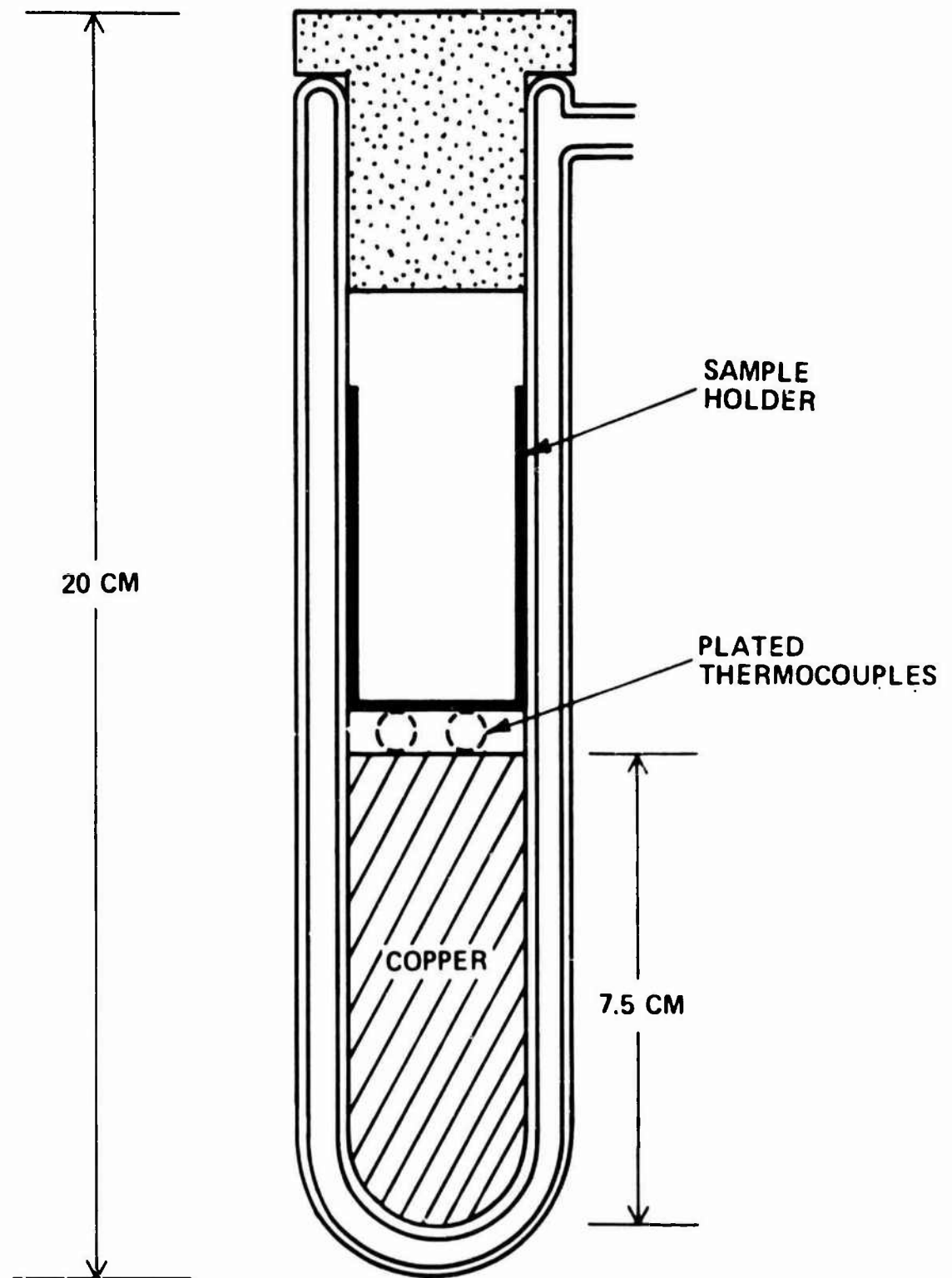


FIG. 11 : DESIGN CONCEPT USING A GLASS DEWAR .

1. The use of a bridge circuit for temperature measurement requires a constant current source. The use of thermocouples will not require this instrumentation.

2. The thermal power produced in the bridge windings is several hundred fold that produced by the bacteria of interest and thus the twin calorimeters at equilibrium are not at the temperature of the surroundings. In this configuration, both the sample chamber and the reference block will be equilibrated to the temperature of the surrounding bath.

3. The use of a thermal reference block rather than an identical second calorimeter retains the advantages of the twin calorimeter with respect to minimizing the impact of bath fluctuations while making the system more compact and easier to assemble. The slow changes in temperature of the reference as heat flows into it from the sample would make absolute calculations of total heats more difficult, but will not significantly affect our detection of initial rates of heat production.

4. With this configuration, the mass of the calorimeter sample chamber can be small compared to the mass of the sample. This will be reflected in a greater temperature change of the calorimeter for small rates of heat production than is found in the Mound Calorimeter.

5. In this configuration when the Dewar is evacuated, we will have a much greater isolation of the calorimeter sample chamber and the thermal reference block from the bath than was achieved in the Mound Calorimeter. This may even result in a reduction in the degree to which a constant temperature must be maintained in the bath.

In our design considerations it appeared advantageous to maintain, if possible, the sample size and sample container used in the present studies, for the following reasons:

1. The sample bottle is commercially available, can be autoclaved, and has been accepted by the microbiologist.

2. It provides a size of sample and container which can be readily manipulated and handled during sample preparation, packaging, and measurement operations.

Thus our initial design concept and calculations are based on use of the Virtis No. 10-156TW-20 bottle with nominal dimensions of 2.86 cm O.D. x 6.15 cm height.

In the course of our studies, several heating curves were obtained in the Mound Calorimeter and in the sample preheater used prior to inserting the sample into the calorimeter. These measurements, in conjunction with the calculations presented in the Appendices, have increased our understanding of the equilibration problem both within the calorimeter sample chamber and between the calorimeter sample chamber and the bath. As discussed in Appendix A when the sample bottle and contents at about 25°C were inserted directly into the calorimeter in equilibrium with the bath at 37°C, it required

4 to 5 hours to reach equilibrium. The temperature vs time curve was an exponential with a time constant of about 22 minutes. When the same bottle and contents were placed in the preheater, an exponential curve was again obtained with a time constant of about 10 minutes; in the preheater; the glass tube containing the bottle plus contents was surrounded directly by the bath, and a calculation assuming that the air gap between the bottle and the glass tube provided the principal thermal barrier gave results agreeing quite well with the experimental results. We also found that if the air gap in the preheater was filled with water, a time constant on the order of 2.6 minutes was obtained; however, the curve no longer was truly exponential indicating that some other barrier which varied with ΔT was becoming important. When convection within the sample in addition to conduction through the water in the original air gap were considered as the primary thermal barriers, a reasonable fit to the experimental results was obtained. Thus three potential barriers of significance to thermal equilibration are indicated as shown in Figure 12. In this figure, we indicate the barrier configuration for radial heat flow and present some relevant thermal conductance data which will be used in assessing the capability of the design concept of Figure 11, or modifications of it, to meet our requirements. From the data presented, we can infer the following:

1. Consider first, equilibration within the sample itself. Calculations show that heat transfer by convection alone varies from $2.3 \text{ W/}^\circ\text{C}$ with a 10°C gradient to $0.16 \text{ W/}^\circ\text{C}$ with a 10^{-4}°C gradient (see Appendix C-1). This lower value is of the same order of magnitude as heat transferred by conduction. When the two are combined, a time constant on the order of 4-5 minutes is indicated for equilibration of the sample under the lowest temperature difference detectable by a system with sensitivity comparable to the Mound Calorimeter used in this work. Such sample equilibration times appear to be adequate for our needs. These calculations are supported by the experimental experience when all barriers to thermal conductance were minimized except that within the sample. These results support the feasibility of the decision to use the same sample size and container as used in the thermal studies reported in this report.

2. Consider next the barrier represented by the air gap between the sample bottle and the sample chamber wall and the question of equilibration within the sample chamber resulting from the combination of thermal barriers. Experiment has shown, and calculation has confirmed that replacing the air with water markedly reduces the importance of this barriers. In fact, as the estimates on Figure 12 show, with water present and for very low values of ΔT , this barrier may be ignored in a first approximation. At large ΔT values, the three barriers under discussion are comparable, but conductance is large, and the resulting time constant for equilibration is extremely small. But we certainly cannot use water in practice to reduce this barrier, and the air gap of 0.047 mm which provides an equivalent conductance would require special machining of sample bottles and chamber which we do not consider desirable. However, in our experiments we did try filling the air gap with metal granules and found time constants intermediate between those found

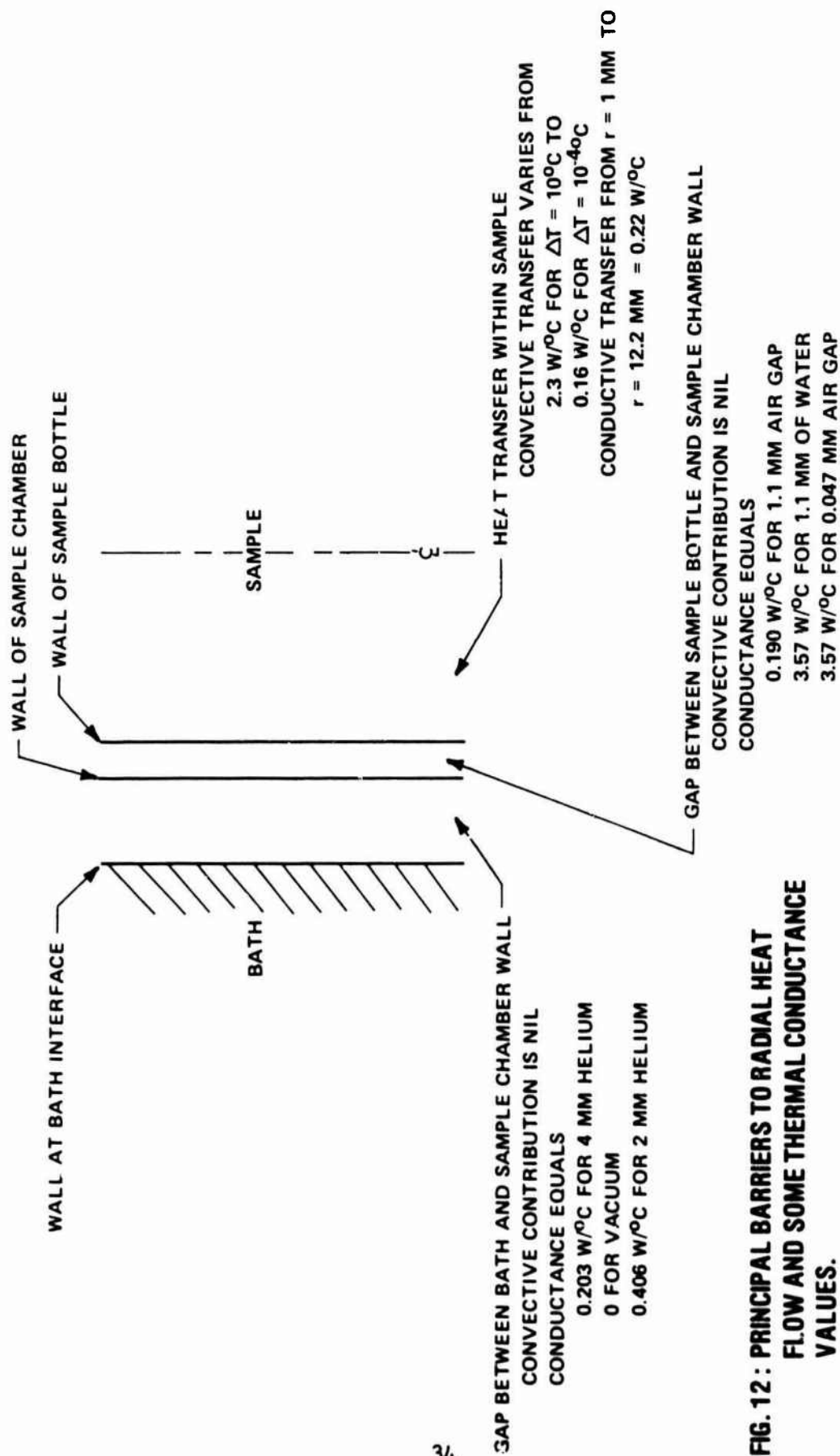


FIG. 12: PRINCIPAL BARRIERS TO RADIAL HEAT FLOW AND SOME THERMAL CONDUCTANCE VALUES.

for air and water. Based on these calculations and experiments, we are reasonably confident that a conductance at least five-fold greater than that for 1.1 mm of air can be achieved by a combination of reducing the air gap two-to-three fold and using a copper foil shim designed to produce a friction fit with many points of contact between the glass bottle and the foil and between the foil and the sample chamber wall. Under these conditions and for a ΔT of 10^{-4}°C across the sample, the conductance within the sample chamber would be on the order of $0.25 \text{ W}/^{\circ}\text{C}$, and the time constant for equilibration would be on the order of 7 minutes. Note that this time is for the lowest temperature gradients of interest and that with higher gradients the convective component within the sample increases, resulting in small time constants over most of the equilibration period. Since the thermocouples are in contact with the sample chamber wall, these calculations are most important in considering the response time of the calorimeter to heat production in the calorimeter; the time constants estimated are considered adequate for our needs.

3. Finally, consider the barrier between the bath and either the sample chamber wall or the copper thermal reference block, and the implications of this barrier on the design. Helium is used instead of air because it provides sixfold more heat transfer. However, a 4-mm gap filled with helium still causes a barrier greater than the worst situation in the sample. If metal construction is used instead of glass, a 2-mm gap can be readily achieved which will give a barrier approximately equivalent to that of the sample under the worst situation. Under these conditions, the time constant is on the order of 13 minutes, and we estimate an average time constant (when recognizing the higher conductances in the sample with higher ΔT) on the order of 10 minutes indicating complete equilibration in under 2 hours as required. However, note that at the beginning of the equilibration, when gradients are large this barrier across the helium is limiting. Additional heat can be introduced directly to the sample chamber wall either by including in the design a resistance heater or by using the thermopile as a heat pump. Such a practice for the first 10-20 minutes should significantly reduce the overall equilibration time. Using the thermopile as a heat pump by passing a current through it would transfer heat from the thermal reference block to the sample chamber wall, but note that the time constant for equilibration of the reference block with only the 2-mm helium space as a barrier is on the order of 6 minutes. Thus the design in Figure 11 with the modifications suggested in these paragraphs appears capable of meeting our needs in terms of achieving equilibration in less than two hours, and it appears to offer the opportunity to achieve equilibrium with the bath in a time much closer to one hour.

This discussion was presented to highlight the significant barriers to equilibration and to emphasize their impact on the final design. Only a radial heat flow situation has been considered. There are additional conductive paths as shown in the Appendices but these do not change the conclusions reached above.

Temperature Measurement and Read Out Instrumentation:

It is intended that the sensitivity of this design meet or exceed that of the Mound Calorimeter. The decision was made to use thermocouples to detect the temperature difference between the sample chamber and the reference block, because this choice appears to allow greater flexibility and simplicity in the overall design.

A construction technique is described in Appendix C-3 which can provide for this design a 250 junction Copper-Constantan thermopile. Using $40\mu\text{V}/^\circ\text{C}$ as the sensitivity of a single thermocouple, this thermopile will have an output of $1\mu\text{V}$ for 10^{-4}°C difference in temperature between the sample chamber and the reference block.

Under steady state conditions with a fixed rate of heat production in the sample chamber, this chamber will lose heat by upward conduction along the tube and through the plug shown on Figure 11 and downward along the tube and through the thermopile assembly. The calculations in the Appendix C-4 for the Figure 11 configuration show that the thermal conductance upward to the bath is $0.0038\text{ W}/^\circ\text{C}$ and that downward to the reference block is $0.0725\text{ W}/^\circ\text{C}$. We will assume that no losses occur through the vacuum and that the thermal reference is at the temperature of the bath. From these calculations and assumptions, we calculate the sensitivity.

$$\frac{(0.0725 + 0.0038)\text{ W}/^\circ\text{C}}{40 \times 10^{-6} \times 250\text{ V}/^\circ\text{C}} = 7.63\text{ W/V} = 7.63 \frac{\mu\text{W}}{\mu\text{V}}$$

The estimated value compares favorably with the value of $18.5\mu\text{W}/\mu\text{V}$ in the Mound Calorimeter and apparently offers considerable leeway for loss of sensitivity due to failure to achieve the degree of isolation believed possible. This sensitivity is not a constant for this design; as the copper reference block heats up due to the heat flowing into it from the sample, this value will change slightly. In Appendix D we show that this factor is not important with respect to our intended use of the instrumentation.

When considering the selection of read-out instrumentation, we must recognize that the final system will include at least 10 calorimeters. The use of expensive microvolt amplifiers such as used with the Mound Calorimeter with each calorimeter would tend to make the total cost prohibitive. On the other hand, the use of switching devices at the microvolt input level is also not considered an inexpensive or necessarily practical approach. The output required by the microbiologist is the thermopile reading (which relates to heat production rate) as a function of time from sample insertion. This output may range from one or less microvolts to several hundred microvolts. However, the initial output or the low end of the range is of primary interest. Thus the introduction of a logarithmic amplifier can allow us to expand a given decade of the output signal without loss of ability to record the higher output signal. This will simplify multichannel recording where each channel may be at a different output level.

The read-out circuitry proposed is shown in Figure 13. Amplifier A-1, an Analog Devices type AD504M, is a low-noise, low-drift, integrated circuit. It is specified to have a maximum input referred noise of 0.6 μ V peak-to-peak, providing useable data down to an input of at least 1 μ V. Analog Devices will be willing, at increased cost, to select these devices for lowest noise (0.3 μ V peak-to-peak, or less, maximum) which would allow good signal conditioning down to inputs of 0.5 μ V or lower from the thermopile.

A-3 is a logarithmic amplifier, Analog Devices type AD 755, which has a four decade range of input voltage, 1.0 mV to 10 V. It conforms to a true logarithmic transfer function within $\pm 1\%$.

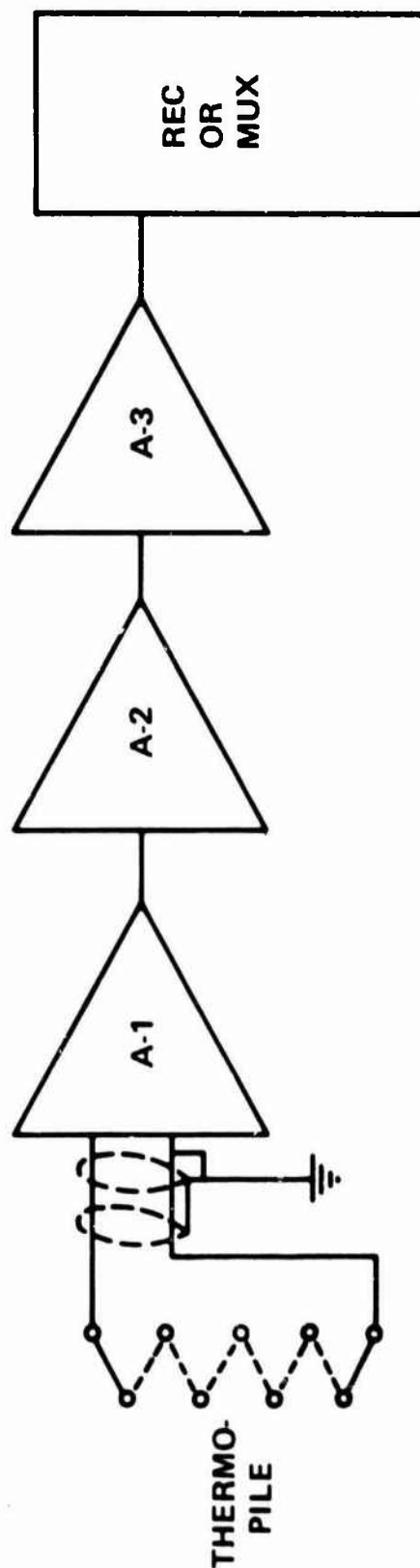
Amplifier A-2 may or may not be needed. The total gain from thermopile to A-3 input must be 10,000 if 0.1 μ V from the thermopile is to provide 1 mV to the logarithmic amplifier. While a gain of 10^4 is realizable with just A-1 (maximum gain specified = 10^6), this might lead to stability problems making necessary the extra stage. Any good amplifier may be used for A-2, since noise will no longer be a problem, but for uniformity another type AD504M, not selected for noise level, is preferred.

The output of the logarithmic amplifier will go to a multi-channel recorder. The scale of this recorder can be selected to cover the number of decades of thermopile output which one wants to record, probably about 2.5 decades or a range of 0.5 to 100 μ V. Drift in this amplifier system will be almost completely eliminated by utilizing the constant temperature bath required for the calorimeter system, to control temperature variations in these units.

Using this system, the maximum cost per channel for microvolt amplification would be approximately \$150:

A-1, Analog Devices AD504M, selected	\$60
A-2, Analog Devices AD504M, non-selected	\$30
A-3, Analog Devices AD755	\$60
Total approximately	<hr/> \$150

Thus the amplifiers for a 10 calorimeter system would be no more costly than the single microvolt amplifier used with the Mound Calorimeter.



A-1 = ANALOG DEVICES AD504M AMPLIFIER, SELECTED
 A-2 = ANY GOOD AMPLIFIER, MAY BE OPTIONAL
 A-3 = ANALOG DEVICES AD755 LOGARITHMIC AMPLIFIER
 REC OR MUX = MULTIPLEXER, SWITCHBOX, OR MULTI-
 CHANNEL RECORDER

FIG. 13: READOUT INSTRUMENTATION

Design Concept

Individual Calorimeter Cell:

The information presented in the previous section on Design Concept Development does, in our opinion, support the feasibility of the configuration suggested for a single calorimeter cell of a multi-cell assay tool. The same configuration adapted to use of metal construction is shown in Figure 14. Metal construction was suggested in the analysis of the previous section as a means of building in a smaller helium barrier during the equilibration period; the smaller gap should be just as effective for purposes of isolation during the heat production measurement period when this gap contain a vacuum. The use of metal construction also eliminates a set of interfaces formed by the inner glass envelope of the Dewar shown in Figure 11; the elimination of such interfaces is desirable to promote heat flow.

This design must be considered as schematic. A final detailed design will consider the use of commercially available construction shapes to minimize shop labor requirements. The only construction aspect for which a design is not well defined is the means to provide a friction fit of the sample bottle into the sample chamber. The objective of providing many metal-conductance paths across the air gap can be achieved by filling the space with granular metal particles or by wrapping the bottle in a dimpled copper foil. However, we are hopeful that a design using rectangular spring elements can be devised which will simplify the introduction and removal of the sample bottle while still achieving a marked reduction in the resistance to heat transfer of the air gap.

Multi-cell layout:

For the first prototype, we propose to use the Tronac isothermal bath and the Tronac model 40 Precision Temperature Controller obtained previously for use with the Mound Calorimeter. A total of ten cells can be readily assembled in this bath. Each cell would be connected through a two-way valve to a vacuum manifold and a helium gas source. The vacuum manifold would be continuously evacuated using a Welch Duo-seal No. 1405 rotary oil pump or its equivalent. Helium would be supplied from a gas cylinder. Thus the space surrounding each sample chamber and separating it from the bath can be either filled with helium or evacuated. The use of a large vacuum manifold is intended to minimize the effect on other cells which are on the vacuum line when an additional one is added; if not successful in practice, an additional roughing pump will be required.

The amplifiers will be mounted on the side of the bath to provide a constant temperature environment and will be thermally insulated from the room.

COMMENTS:

1. INNER SURFACES OF DEWAR SILVERED TO REDUCE RADIATION TRANSFER.

2. THREADED STAINLESS STEEL PINS PROVIDING TURNBUCKLE ACTION CAN POSITION AND CONTROL DISTANCE OF COPPER THERMAL REFERENCE BLOCK WITH RESPECT TO SAMPLE CHAMBER.

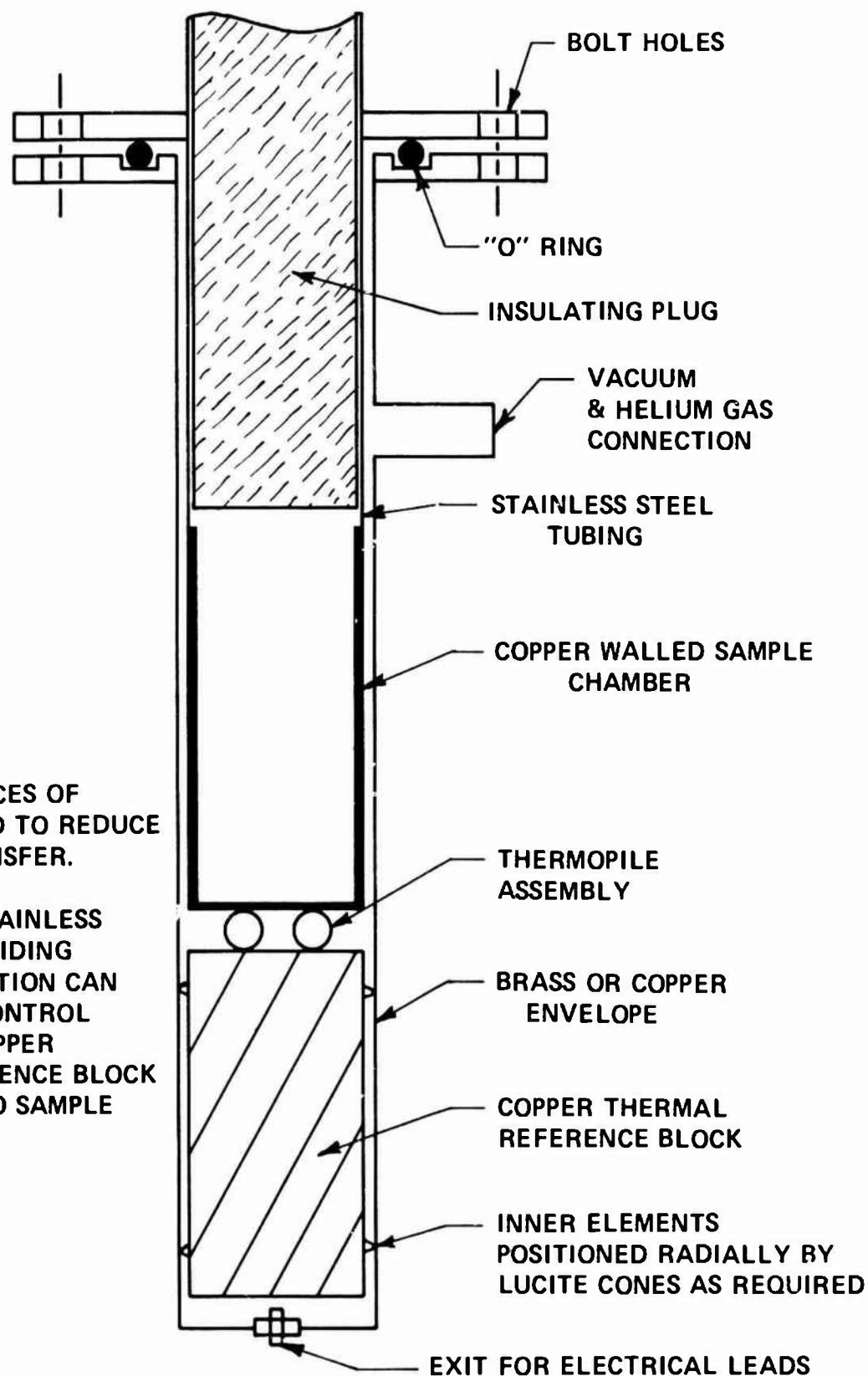


FIG. 14 : CONCEPT FOR CALORIMETER BASED ON METAL CONSTRUCTION.

System Cost Estimates:

The total cost of such a calorimetric instrument in production lots of ten or so is not easy to assess at this stage of development. Cost of building and assembling the system can be better defined after the design has been fixed in all details and a prototype system has been built. However, some estimates can be given for the equipment which must be purchased as shown in Table IV.

Table IV

Procurement Cost Estimates for a Ten Sample Instrument

Constant Temperature Bath & Controller	\$1600
10 Amplifiers, selected	600
10 Amplifiers, non-selected	300
10 Logarithmic Amplifiers	600
1 Multi-point Recorder	3000
1 Vacuum Pump (Welch Duo-Seal 1405)	300
Equipment Sub-Total	<hr/> \$6400

This listing gives a purchased equipment cost of \$6400. The remainder of the cost would be supplies, materials, construction, and assembly costs. At this stage of development, we can only guess that these costs might run from 5 to 10 thousand dollars. This would imply a system cost on the order of \$15,000 for a tool that would be used daily to analyze food samples for microbial contamination in a central food preparation facility. We must emphasize again that we have no good basis for estimating the labor cost for construction and assembly, but this exercise at least shows that we cannot get such an instrument for \$5000 and probably will not have to pay \$20-25,000.

Conclusions

1. Heat production data were determined as a function of concentration for three aerobic mesophilic bacteria selected as representative of those to be found on contaminated foodstuff. These data were determined at the temperature (37°C) and in a growth medium known to provide rapid growth for such bacteria. The results indicate that to detect contamination levels of 10^5 bacteria per gram in food samples, a calorimeter with a sensitivity at least equal to that used in the studies ($18.5 \mu\text{W}/\mu\text{V}$) is required together with a capability to record meaningful heat output data by two hours after sample introduction. With such equipment, measurable heat outputs observed in 3 hours or less would indicate a probability that the food sampled had contained 10^5 or greater bacterial count per gram. These results are within the desired one- to four-hour detection time as discussed in the introduction; they indicate that test times for a calorimeter assay technique may be as short as any other technique currently under study. As discussed in the text, different growth media can give higher heat outputs, and the opportunity exists to improve the calorimeter sensitivity; therefore, it may be possible to reduce the 2 to 3 hour detection period.

2. Calorimeter design requirements were further defined through experiment and calculation. A design concept for an individual calorimeter unit is presented together with performance estimates indicating that the required sensitivity and equilibration time requirements can be met and very likely exceeded. Readout instrumentation requirements and the layout for a ten sample system are described. Potential system cost estimates are considered.

Recommendations

1. Proceed with the detailed design, construction, assembly, and test of a single calorimeter unit. The design effort must include determination of the details of a "friction fit" design for the sample bottle in the sample chamber so that the conductance obtained will be several fold greater than that using a loose fit with an air gap.

2. Adopt a final unit design and build a multi-sample assay tool for use by the microbiologists in fully assessing the potential for this assay technique in detecting microbial contamination of foodstuff. The assessment should include the evaluation of alternative growth media which may provide higher levels of heat production and thus further decrease the time required for detection of microbial contamination.

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APPENDIX A

Appendix A

Heat Production Calculation from Calorimetric Data

The heat production in the sample is given by the expression

$$P = k_1 \Delta T + \frac{d}{dt} (C\Delta T)$$

where k_1 is the constant relating the rate of heat flow from the calorimeter to the temperature difference between the twin calorimeters, and C is the per degree heat capacity of the calorimeter plus sample. The measured voltage signal from the bridge, v , is proportional to ΔT , so we may define the constants:

$$\Delta T = k_3 v$$

and $k_1 \Delta T = k_2 v$

The constant k_3 may be calculated from the temperature coefficient of resistance (α) of the wire (pure nickel) used in the bridge, the measured resistance of a bridge arm (R) and the current (i) maintained to the bridge.

$$v = i/2 \Delta R = i/2 \alpha R \Delta T$$

$$v/\Delta T = i/2 \alpha R = 0.005 \times \frac{0.00575}{2} \times 1417 \text{ V}/^\circ\text{C}$$

$$\text{and } k_3 = \frac{\Delta T}{v} = 4.909 \times 10^{-5} \text{ }^\circ\text{C}/\mu\text{V}$$

The constant k_2 is determined from calibration of the calorimeter by providing a known power input electrically, using the heater in the calorimeter, and observing the signal, v , at equilibrium where $d/dt (C\Delta T) = 0$.

$$\text{Then } k_2 = P/v = 18.5 \text{ } \mu\text{W}/\mu\text{V} \text{ (from measurements) and of course } k_1 = k_2/k_3.$$

The value of C is determined by observing the cooling rate of the calorimeter. Since the heat content which is to be determined is a rather indeterminate mass including the sample and a good bit of the inside of the calorimeter, it is important that we determine C under the same conditions for which it is to be used; that is, with the sample bottle as the source of heat. So a slightly warmed, sterile bottle is placed in the calorimeter and the cooling is observed. Here $P = 0$, and if we designate T_0 as the starting temperature, and T_A as the ambient,

$$k_1 \Delta T = - \frac{d}{dt} (C\Delta T)$$

$$\frac{k_1}{C} dt = - \frac{dT}{T - T_A}$$

$$\frac{k_1}{C} t = \int_{T_0}^T \frac{dT}{T - T_A} = - \ln \frac{T - T_A}{T_0 - T_A}$$

$$(T - T_A) = (T_0 - T_A)e^{-t/\tau}$$

$$\text{or } \Delta T = \Delta T_0 e^{-t/\tau}$$

where $\tau = C/k_1$, the time constant.

Implicit in this analysis is the assumption that there is a uniform temperature, T , of a mass, with a heat content, C , on one side of a heat barrier and a constant temperature, T_A , on the other side. This can't be strictly true since there is a distribution of temperature in the bottle, and part of the material contributing to the value of C is also part of the heat barrier. The latter point is taken care of if C is determined as described. The impact of temperature distribution in the sample will also be minimized if all heat flow processes are determined by constants which are essentially independent of the temperature difference, as is expected for conduction. However, there can be convective heat transfer in the bottle and in the air around the bottle, and convection is very dependent on temperature gradients. In Appendix C-1 we show that convection in the bottle can be an important process of heat transfer in the sample and the convection in the air gap between the bottle and the chamber wall is not an important process. However, tests comparing bottle heating in the calorimeter with heating in the preheater show that processes in the calorimeter are principally determined by the heat barrier or thermal resistance of the calorimeter. Thus the present analysis should be essentially correct. This will be confirmed if the time constant determined as above is truly a constant.

The results of such a cooling test are shown in Fig. A1. There was sufficient sensitivity to determine four time constants. The value of the constant did change somewhat, but should be constant enough for our purposes, and has a value of about 26 minutes. The constant k_2 was measured to be $18.5 \mu\text{W}/\mu\text{V}$ so

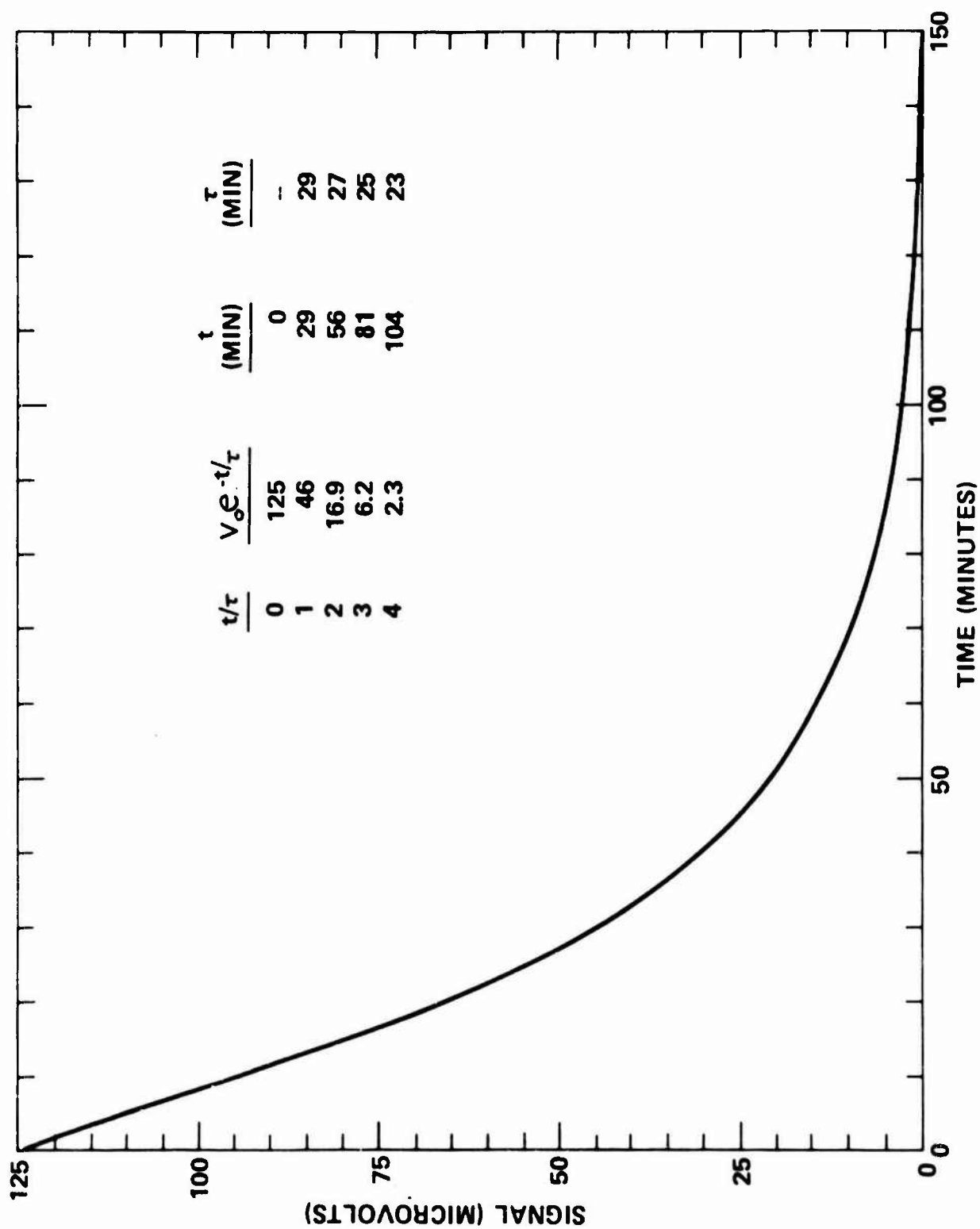


Fig. A-1 : COOLING CURVE IN MOUND CALORIMETER.

$$k_1 = k_2/k_3 = \frac{18.5}{4.909 \times 10^{-5}} = 3.77 \times 10^5 \mu\text{W}/^\circ\text{C} = .377 \text{ W}/^\circ\text{C}$$

$$C = k_1 \tau = 0.377 \times 26 \times 60 = 588 \text{ J}/^\circ\text{C}$$

$$\begin{aligned} \text{so } P &= k_2 v + k_3 C dv/dt \\ &= 18.5 v + 8.02 (dv/dt) \end{aligned}$$

with P in μW , v in μV and dv/dt in $\mu\text{V/hr}$.

This equation was used to derive the heat production data in Figures 7-9 of the text from v and dv/dt values taken from the calorimetry measurements in Figures 1-6.

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APPENDIX B

Appendix B

An Analysis of the Bottle Heating Problem

In a conduction calorimeter, heat produced in the sample passes through a barrier to a heat sink. The temperature drop across this barrier is a measure of the heat flow and so of the rate of heat production. In order to measure small rates of heat production in the sample calorimeter, one must be able to measure small temperature differences between the sample and the sink or, in the case of the Mound Calorimeter, between the twin calorimeters. With the Mound Calorimeter used in this work, a detectable signal of one microvolt corresponds to a temperature difference of $5 \times 10^{-5}^{\circ}\text{C}$. Obviously, before any meaningful measurements of small heat production levels of the sample may be made, the sample must be equilibrated to a temperature difference less than this.

If a sample bottle at room temperature, say 27°C , is placed in the Mound Calorimeter operating at 37°C , it takes 2 hours to reach a ΔT of 0.05°C and about another $2\frac{1}{2}$ hours to get down to $5 \times 10^{-5}^{\circ}\text{C}$. In order to shorten this time we preheat the bottle. The first two or three hours of equilibration in the calorimeter are speeded up by use of a preheater with a greater rate of heat exchange, but the exact speed and manner of transferring from the preheater to the calorimeter has been too great a variable to accomplish equilibration to closer than a few hundredths of a degree in a preheater.

The preheater used was a glass jacket with a cavity about the same diameter as the calorimeter. Water at a controlled temperature was circulated in this jacket. This system heated the bottle a little more than twice as fast as in the calorimeter, which still isn't as fast as we would like to achieve directly in the calorimeter. So the problem is how to heat the sample bottle directly in a calorimeter and even faster than was done in the preheater.

To better understand the equilibration problem we will attempt to calculate heat flows and relate these data to experience. In the pre-heater the circulating water is maintained at a constant temperature. When a cold bottle is inserted, heat is conducted through the glass wall; this heat is transferred from the wall to the bottle by conduction and/or convection in the air, by radiation, and by conduction at points of contact between the wall and bottle; it is then conducted through the glass of the bottle and finally transferred to the liquid, principally by convection. If we can calculate each of these processes and combine their overall effect, we can compare calculated heating rates with measured rates and so gain confidence in our calculations for a design where we can't make measurements.

For each process of heat transfer, we wish to determine a heat transfer coefficient, h , defined by the relation

$$P = hA\Delta T$$

where P is the rate of heat transfer for a temperature difference, ΔT , and an area, A . The quantity h is most useful when it doesn't vary with temperature or temperature difference. The heat transfer coefficient for conduction varies very slowly with temperature and for our purposes may be considered constant. Radiative transfer varies with the fourth power of the absolute temperature, but for the comparatively small values of ΔT encountered here an h for radiation can be derived as shown later in this discussion which doesn't change significantly. Thus in a total heat transfer situation in which convection contributes little we have a constant h for the entire process. However, the convective heat transfer coefficient normally depends strongly on ΔT , and when convection is an important component of the total heat transfer process, we cannot use a constant value of h , but must use a calculation method which takes into consideration the temperature gradient in the convective medium. These comments are expanded upon in the following discussion.

If h is essentially a constant for the overall process, the heating is exponential. This is shown as follows:

$$hA\Delta T = C_s \frac{dT}{dt} \quad \text{where } C_s \text{ is the heat capacity of the sample being heated}$$

$$t = \frac{C_s}{hA} \int_{T_0}^T \frac{dT}{T_a - T} \quad \text{where } T_0 \text{ is the starting temperature of the sample and } T_a \text{ is the temperature of the surroundings}$$

$$t/\tau = -\ln \frac{T_a - T}{T_a - T_0} \quad \text{where } \tau = C_s/hA$$

$$\frac{T_a - T}{T_a - T_0} = e^{-t/\tau}$$

$$\Delta T = \Delta T_0 e^{-t/\tau} \quad \text{where } \Delta T \text{ is the temperature difference at time } t \text{ and } \Delta T_0 \text{ that at time zero}$$

Thus if h is a constant, we can calculate the time constant and use it to compare the heating under different conditions. The heating behavior observed in the Mound Calorimeter from a ΔT of 10°C to one of 0.05°C in 2 hours gives a time constant of 22.6 minutes; from 10°C to $5 \times 10^{-5}^\circ\text{C}$ in $4\frac{1}{2}$ hours corresponds to a time constant of 22.1 minutes. Thus our observations indicate that this process is exponential. If we wish to develop a system that would accomplish this equilibration in one hour, it should have a time constant of about five minutes, since equilibration from a ΔT of 10°C to about 10^{-4}°C requires a total time of about 10τ .

We next return to calculations for heating in the preheater; for conduction in air at 37°C , $k = 2.69 \times 10^{-4} \text{ W/cm}^\circ\text{C}$, and the air gap around the bottle is about 1.1 mm. So for conduction:

$$h_c = \frac{k}{\ell} = \frac{2.69 \times 10^{-4}}{0.11} = 2.45 \times 10^{-3} \text{ W/cm}^2^\circ\text{C}$$

Radiative heat transfer may be expressed as:

$$q/A = \epsilon \sigma (T_a^4 - T^4)$$

where ϵ is the emissivity and σ is the Stefan-Boltzman constant.

$$\sigma = 5.67 \times 10^{-12} \text{ W/cm}^2 \text{K}^4$$

There is a more exact equation for radiative transfer between one cylinder inside another such as we have here, but for values of ϵ near one it doesn't make much difference. Since we have no surfaces either very shiny or very dull, an ϵ of 0.90 is a good approximation. In order to get a heat transfer coefficient with the same units as for conduction we divide by ΔT .

$$h_r = \epsilon \sigma \left(\frac{T_a^4 - T^4}{T_a - T} \right)$$

For a ΔT of 10°C and a T_a of 37°C we have

$$h_r = \frac{0.90 \times 5.67 \times 10^{-12} (310^4 - 300^4)}{10} = 5.79 \times 10^{-4} \text{ W/cm}^2^\circ\text{C}$$

For a ΔT of 1°C this has risen to 6.05×10^{-4} and as ΔT vanishes it approaches 6.08×10^{-4} . So we will have very little error if we use a constant value of

$$h_r = 6 \times 10^{-4} \text{ W/cm}^2^\circ\text{C}$$

The applicable area for h_c and h_r is the total outside area of the bottle.

$$A = \pi \times 2.86 \times 5.5 + 2 \times \frac{\pi}{4} \times 2.86^2 = 62.3 \text{ cm}^2$$

Convection is not appreciable under these conditions (see Appendix C-1). The total heat transfer will be affected somewhat by conduction in the glass of the preheater and bottle and by convection in the liquid but the effect will be small and not included here. So for the total heat transfer per unit ΔT

$$\begin{aligned} hA &= h_c A + h_r A \\ &= 2.45 \times 10^{-3} \times 62.3 + 6 \times 10^{-4} \times 62.3 \\ &= 0.190 \text{ W}^\circ\text{C} \end{aligned}$$

The sample bottle contains 24 g of liquid, basically water. The glass bottle has a water equivalent of 4 g, so

$$\begin{aligned} C_s &= 28 \text{ g} \times 4.184 \text{ J/g}^\circ\text{C} = 117.2 \text{ J}^\circ\text{C} \\ \text{and } \tau &= \frac{C_s}{hA} = \frac{117.2}{0.190} = 618 \text{ sec} = 10.3 \text{ min} \end{aligned}$$

When a bottle was heated in the preheater, the time constant obtained was about 9.5 min. This is probably as close an agreement as can be expected for a calculation of this kind.

In order to reduce this time constant, one means to consider is the reduction of the dimension of the air gap; that is, make the bottle a better fit in the cavity. However, when this barrier to heat flow is reduced, we can no longer use the same method for our calculations, because quantities we have up to now ignored become appreciable. This is apparent from heating curves obtained in the preheater with the bottle surrounded with water.

For this situation we find that we must consider heat transfer through the preheater glass, the water in the former air gap, and the sample bottle glass, and finally, the distribution of heat into the sample in the bottle by convection. This last transfer is the most complicated to calculate. However, it is important, as we find out if we try a calculation ignoring it. Such a calculation gives a time constant of about 1.5 minutes. The observed heating curve does not quite follow an exponential law, but an average of the first four time constants, computed as if it was an exponential curve, gives a value of 2.6 minutes. This lack of agreement indicates neglect in our calculations of a significant barrier to heat transfer. This is convection in the sample, which is analyzed in Appendix

C-1. We see from this analysis that the thermal conductance for the convective process of heat transfer, H_v , in the sample is a function of ΔT . This explains why the heating curve observed is not exponential. The following scheme can be used to calculate a heating curve which considers the convective component of heat transfer.

The ΔT referred to in Appendix C-1 is not the total ΔT that is measured in our heating curve experiment, but it is only the temperature drop across the convection boundary layer. We shall call this ΔT_v . The method of calculation requires that we start by assuming a ΔT_v , determine H_v from the curve given in Appendix C-1 and calculate ΔT as indicated below. In order to get a particular overall ΔT , say of 10°C , a trial and error process is necessary. To calculate ΔT we note that the heat flux through the convection layer must equal the total flux; this is true over practically the total calculation since it is only at the lowest ΔT values of interest that the convective transfer component approaches the low value of the conduction component.

$$\begin{aligned} H_v \Delta T_v &= H_t \Delta T \\ \Delta T &= \frac{H_v \Delta T_v}{H} = H_v \Delta T_v \left(\frac{1}{H_{p.g.}} + \frac{1}{H_w} + \frac{1}{H_{b.g.}} + \frac{1}{H_v} \right) \\ &= \Delta T_v \left[H_v \left(\frac{1}{H_{p.g.}} + \frac{1}{H_w} + \frac{1}{H_{b.g.}} \right) + 1 \right] \end{aligned}$$

where $H_{p.g.}$, H_w , $H_{b.g.}$ are the thermal conductances across the preheater glass, water layer and bottle glass. We can also solve for the total conductance, H_t , and can then write the equation:

$$H_t \Delta T = C_s \frac{dT}{dt}$$

where C_s , the heat capacity, has the same value as before, $117.2 \text{ J}^\circ\text{C}$. Since H_t is a function of ΔT we cannot solve this equation directly but since we know their values at any time we can calculate the average values over an interval and use this with little error. So we write:

$$dt = \frac{C_s}{H_t \text{ ave}} \frac{dT}{\Delta T \text{ ave}}$$

Here dt and dT are taken as finite intervals of time and temperature. By adding successive values of dt we calculate the heating as tabulated in Table B-1. Each heat transfer coefficient derived earlier is applied to the appropriate area to calculate thermal conductances; that is, for the preheater glass — the surface in the region of the bottle; for the water and bottle glass — the outer surface of the bottle; and for the convection — the vertical surface in the bottle. For glass $k = 0.011 \text{ W/cm}^\circ\text{C}$ and $\ell = 0.15 \text{ cm}$ and for water $k = 0.00623 \text{ W/cm}^\circ\text{C}$ and $\ell = 0.11 \text{ cm}$. We also include radiation at this point though it contributes little. The thermal conductances are:

$$H_{p.g.} = \frac{Ak}{\ell} = \frac{54.3 \times 0.011}{.15} = 3.98 \text{ W}^\circ\text{C}$$

$$H_{b.g.} = \frac{AK}{\ell} = \frac{62.3 \times 0.011}{0.15} = 4.57 \text{ W}^\circ\text{C}$$

$$H_w = A \left(\frac{k}{\ell} + h_r \right) = 62.3 \left(\frac{0.00623}{0.11} + 0.0006 \right) = 3.57 \frac{\text{W}}{^\circ\text{C}}$$

With these data we find

$$\frac{1}{3.98} = \frac{1}{4.57} = \frac{1}{3.57} = 0.750$$

$$\text{so } \Delta T = \Delta T_v (0.750 H_v + 1) \text{ where } H_v = 42.6 h_v$$

$$\text{and } H_t = \frac{1}{0.750 + 1/H_v}$$

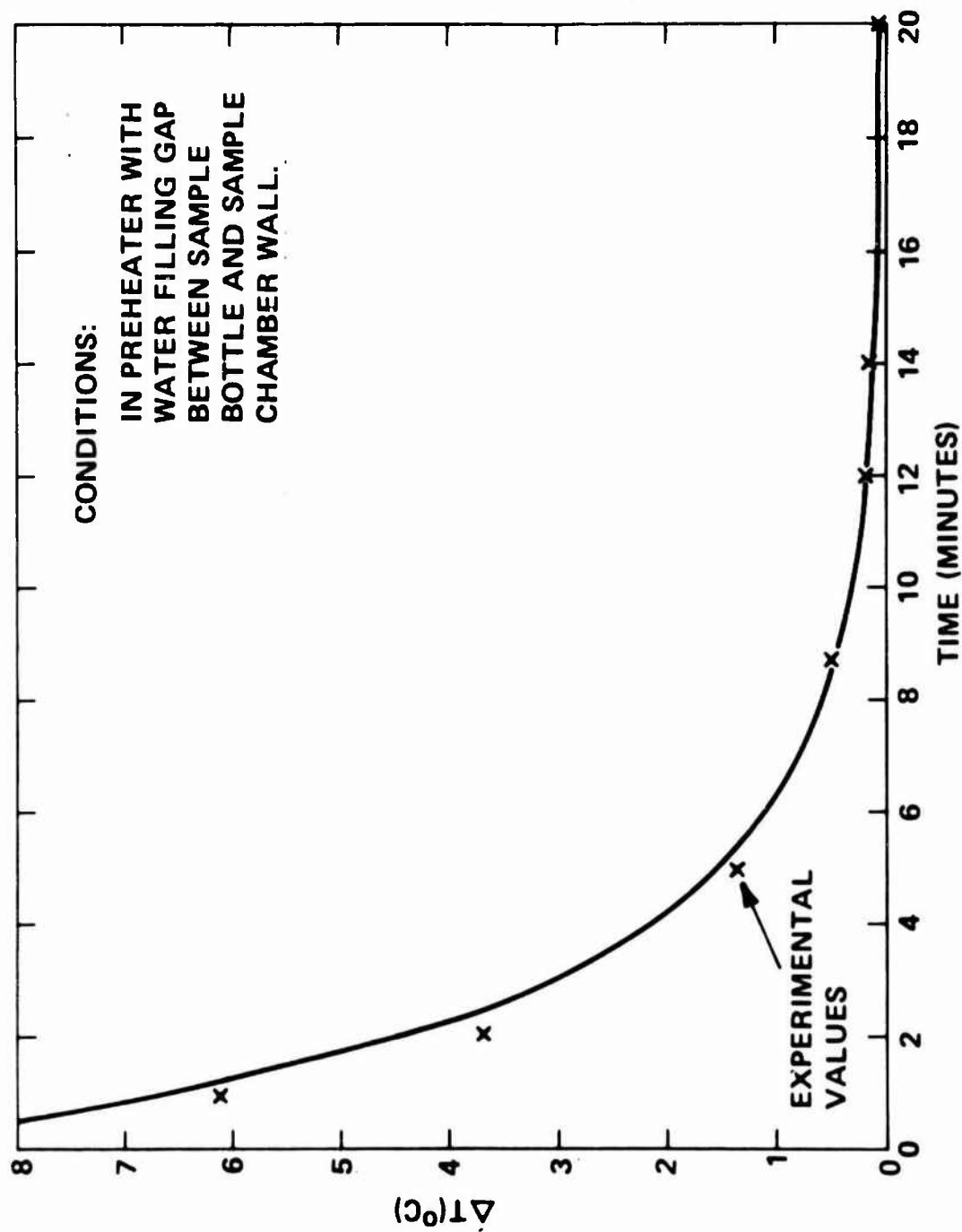
In Fig. B-1 we plot a curve from Table B-1 for ΔT vs time, together with some measured values. The measured values were made with a single thermocouple, and therefore are not taken to extremely small ΔT values, but as far as they go, the agreement is good. The calculations weren't taken down to the ΔT of $5 \times 10^{-5}^\circ\text{C}$ we want to reach in the calorimeter, but it is estimated that this temperature could be reached in a little over an hour which is the time scale we are trying for.

With air outside the bottle, we must reduce ℓ to achieve a conductance comparable to the water. We can calculate what it must be in order to give the same times as the above calculation.

TABLE B-1

Estimate of Heating Curve in Pre-Heater with Water in Gap between
Sample Bottle and Chamber Wall

ΔT_v (°C)	H_v (W/°C)	ΔT (°C)	H_t (W/°C)	H_t ave (W/°C)	dT (°C)	ΔT ave (°C)	dt (sec)	t (sec)	t (min)
4.1	1.92	9.99	0.787	—	—	—	—	0	0
3.0	1.75	6.94	0.757	0.772	3.05	8.46	54.7	54.7	0.91
2.0	1.58	4.38	0.723	0.740	2.56	5.66	71.6	126.0	2.1
1.0	1.33	2.00	0.666	0.695	2.38	3.19	125.8	252.0	4.2
0.5	1.13	0.92	0.612	0.639	1.08	1.46	136.0	388.0	6.5
0.3	1.00	0.53	0.571	0.592	0.39	0.725	106.0	494.0	8.2
0.1	0.75	0.16	0.480	0.526	0.37	0.345	239.0	733.0	12.2
0.05	0.626	0.073	0.426	0.453	0.087	0.116	194.0	927.0	15.5
0.01	0.426	0.013	0.323	0.375	0.06	0.043	436.0	1363.0	22.7
0.005	0.362	0.0064	0.285	0.304	0.0066	0.0097	262.0	1626.0	27.1
0.001	0.298	0.0012	0.244	0.265	0.0052	0.0038	605.0	2231.0	37.2
0.0005	0.247	0.00059	0.208	0.226	0.00061	0.0009	351.0	2582.0	43.0
0.0001	0.162	0.00011	0.144	0.176	0.000488	0.00035	913.0	3496.0	58.3



**FIG. B-1 : CALCULATED HEATING CURVE (INCLUDING CONVECTION
COMPONENT IN SAMPLE) COMPARED TO EXPERIMENTAL DATA**

$$h_{\text{air}} = h_{\text{water}} = \frac{k_{\text{air}}}{\ell_{\text{air}}} = \frac{k_{\text{water}}}{\ell_{\text{water}}}$$

$$\ell_{\text{air}} = \frac{k_{\text{air}}}{k_{\text{water}}} \times \ell_{\text{water}} = \frac{2.69 \times 10^{-4}}{0.00623} \times 0.11 = 0.0047 \text{ cm}$$

It is difficult to say whether this can be achieved or not, but it seems that it would not be possible with ordinary molded bottles. It would require that the bottles be ground to size and the cavities, machined to size.

Finally we are in position to calculate bottle heating for the design of Figure 11 presented in the text. In operation, the vacuum space surrounding the calorimeter during measurement is to be flooded with Helium or mercury during the equilibration period. If it is necessary to use mercury, we will have the question of whether we can get a good enough vacuum with continuous pumping when we need it. For calculating bottle heating, conductance in this helium or mercury filled space together with the surrounding glass takes the place of that in the preheater glass in the above calculation for bottle heating in the preheater. In Appendix C-2 we calculate the total heat transfer coefficient through this space with either helium or mercury. We find that it is 0.250 W/°C with helium, 1.70 W/°C with mercury, either of which will replace the value of 3.98 W/°C for the preheater glass used in the calculation above. We assume that the metal sample chamber is a perfect conductor and that it is machined and the bottles ground to give the same heat transfer coefficient across the air gap as in the preheater with water. If this is not possible, it might be possible to use a liquid around the bottle in the calorimeter. If we used water it would be necessary to seal the cavity to prevent any heat absorbing evaporation. However, a low vapor pressure liquid could be used without sealing, or we could use a powdered metal. Any of these alternatives appears to complicate use of the system, so it will be much better if we can use either precision spacing or develop a friction fit alternative that significantly reduces the magnitude of the air gap barrier.

So, replacing just the coefficient corresponding to the preheater glass, we proceed as above and find that to reach the ΔT which took just under an hour with the preheater requires over two hours with helium and just over an hour with mercury. These calculations are tabulated in Tables B-2 and B-3.

TABLE B-2
Estimate of Heating Curve in Design Concept with Helium Surrounding Calorimeter Cell
and Small Air Gap Around Sample Bottle

ΔT_v (°C)	H_v (W/°C)	ΔT (°C)	H_t (W/°C)	$H_{t \text{ ave}}$ (W/°C)	dT (°C)	$\Delta T \text{ ave}$ (°C)	dt (min)	t (min)	t (hours)
1.35	1.44	10.07	0.1925	—	—	—	—	—	—
1.0	1.33	6.98	0.190	0.1913	3.09	8.525	3.70	3.70	0.062
0.7	1.23	4.58	0.188	0.189	2.40	5.78	4.29	7.99	0.133
0.4	1.07	2.32	0.184	0.186	2.26	3.45	6.88	14.87	0.248
0.2	0.92	1.02	0.179	0.1815	1.30	1.67	8.38	23.2	0.39
0.1	0.75	0.435	0.171	0.175	0.585	0.7275	8.98	32.2	0.54
0.05	0.626	0.191	0.164	0.1675	0.244	0.313	9.09	41.3	0.69
0.03	0.545	0.104	0.158	0.161	0.087	0.1475	7.16	48.5	0.81
0.005	0.362	0.0130	0.138	0.142	0.016	0.021	10.5	73.4	1.22
0.001	0.298	0.0023	0.127	0.1325	0.0107	0.00765	20.6	94.1	1.57
0.0001	0.162	0.00017	0.094	0.1055	0.00093	0.000635	27.1	132.5	2.2

TABLE B-3

Estimate of Heating Curve in Design Concept with Mercury
Surrounding Calorimeter Cell and Small Air Gap Around Sample Bottle

ΔT_v (°C)	H_v (W/°C)	ΔT (°C)	H_t (W/°C)	$H_{t\text{ ave}}$ (W/°C)	dT (°C)	$\Delta T_{\text{ ave}}$ (°C)	dt (sec)	t (sec)	t (sec)
3.4	1.81	9.93	0.621						
2.5	1.67	6.92	0.604	0.6125	3.01	8.425	68.4	68.4	1.14
2.0	1.58	5.34	0.591	0.5975	1.58	6.13	50.6	118.9	1.98
1.5	1.47	3.84	0.575	0.583	1.50	4.59	65.7	184.6	3.08
1.0	1.33	2.41	0.553	0.564	1.43	3.125	95.1	279.7	4.66
0.5	1.13	1.10	0.515	0.534	1.31	1.755	163.8	443.5	7.39
0.3	1.00	0.62	0.486	0.5005	0.48	0.86	130.7	574.2	9.57
0.1	0.75	0.179	0.418	0.452	0.441	0.3995	286.2	860.5	14.3
0.5	0.826	0.083	0.377	0.3975	0.096	0.131	216.1	1076.5	17.9
0.01	0.426	0.0145	0.294	0.3355	0.0685	0.04875	490.9	1567.4	26.1
0.005	0.362	0.0069	0.262	0.288	0.0076	0.0107	289.0	1856.3	30.9
0.001	0.298	0.0013	0.227	0.2445	0.0056	0.0041	655.0	2511.0	41.9
0.0005	0.247	0.00063	0.196	0.2115	0.00067	0.000965	385.0	2896.0	48.3
0.0001	0.162	0.00012	0.138	0.167	0.00051	0.000375	954.0	3850.0	64.2

APPENDIX C

Appendix C

Heat Transfer Calculations

1. Free Convection

Convection in either a liquid or a gas may be calculated from fundamental quantities in only a very limited number of simple cases. However, much experimental work has been done, and this can usually be correlated by the use of various dimensionless quantities. These are:

the Prandtl number, $Pr = \frac{C_p \mu}{k}$

where C_p is the specific heat at constant pressure
 μ is the dynamic (or absolute) viscosity
and k is the thermal conductivity,

the Nusselt number, $Nu = \frac{h \cdot x}{k}$

where h is the heat transfer coefficient
and x is a characteristic dimension

and the Grashof number, $Gr = \frac{g \beta p^2 \Delta T x^3}{\mu^2}$

where g is the acceleration of gravity,

β is the volume coefficient of expansion
 p is the density

and ΔT is the temperature difference causing the convection.

First we shall consider the possibility of convection in the 1.1-mm air gap between the preheater wall and the sample bottle. It can be shown* for enclosed air spaces between vertical walls that if the Grashof number is less than 2000, convection is not significant compared to conduction. For the case of air at 37°C (310K)

* Jakob, M.: Free convection through enclosed plane gas layers, *Trans ASME*, vol 68, p 189, 1946.

$$\rho = 11.38 \times 10^{-4} \text{ gm/cm}^3$$

$$\mu = 1.89 \times 10^{-4} \text{ gm/cm sec}$$

$$\beta = 3.23 \times 10^{-3} \text{ K}^{-1} \text{ (for gases } \beta = 1/T)$$

$$g = 980 \text{ cm/sec}^2$$

The space in question is 0.11 cm across, and the greatest ΔT we would have would be 10°C ;

so $Gr_{\text{air}} = 1.5$, indicating that convection transfer in the air gap is not significant.

if we repeat for water in this gap at 37°C ,

$$\rho = 0.9954 \text{ g/cm}^3$$

$$\beta = 0.000286 \text{ K}^{-1}$$

$$\mu = 0.006915 \text{ g/cm-sec}$$

so $Gr_{\text{water}} = 77$, indicating again that we can ignore convection in this space.

However, this is not the situation for the liquid inside the bottle. As was discussed in Appendix B, it is necessary to include the convective transfer component as developed below in order to explain not only the observed rates of heating but also the lack of exponential heating behavior under some conditions. Most of the experimental work in natural convection can be correlated by equations of the form

$$\overline{Nu} = \frac{\bar{h}_x}{k} = C (Gr Pr)^m$$

where the bars over the quantities refer to the average value over the surface. The constants C and m depend on the geometry and range of properties, C usually being between 0.1 and 0.6, and m either $1/4$ or $1/3$. For instance, for vertical planes or cylinders when the product $Gr Pr$ is between 10^4 and 10^9 the equation is:

$$\overline{Nu} = 0.59 (Gr Pr)^{1/4} \cdot$$

* McAdams, W. H., Heat Transmission, 3rd ed. McGraw-Hill Book Co., New York, 1954.

To calculate Gr Pr we need for water at 37°C, besides the values given earlier,

$$C_p = 4.178 \text{ J/g}^\circ\text{C}$$

$$k = 0.00623 \text{ W/cm}^\circ\text{C}$$

and the height of the effective cylindrical surface on the bottle, 5 cm, as the characteristic dimension X.

$$\text{Gr Pr} = \frac{C_p g \beta \rho^2 x^3 \Delta T}{\mu k} = 3.36 \times 10^6 \Delta T$$

So we see that for much of the bottle heating situation we are investigating we are in the range of the above equation, but before we get down to $\Delta T = 5 \times 10^{-5}^\circ\text{C}$ the Gr Pr product will be below 10^4 . In this low region a slightly different, graphical scheme must be used, the details of which we need not go into here. (see McAdams, 1954)

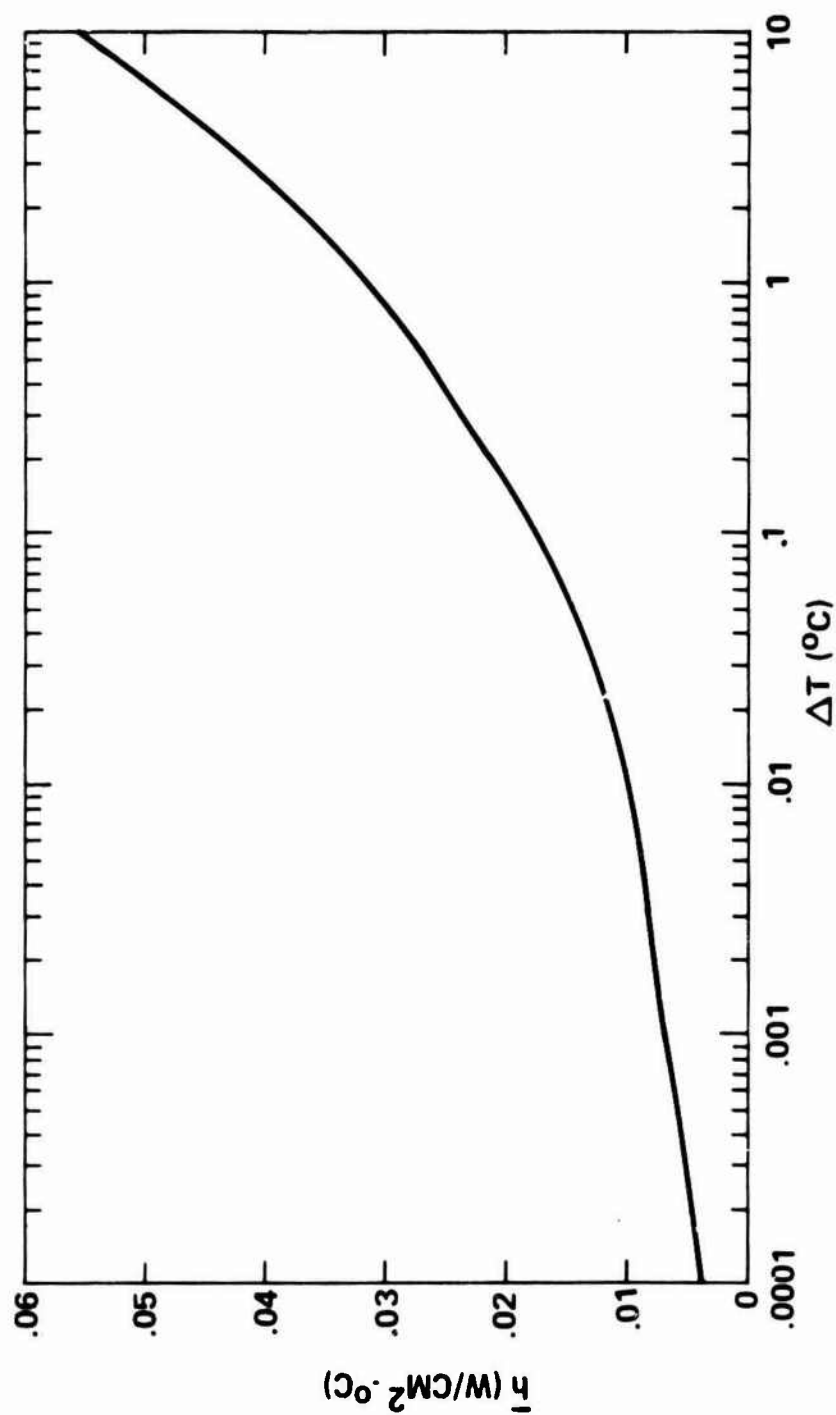
So we have:

$$\bar{h} = \frac{k}{x} \quad \overline{\text{Nu}} = 7.35 \times 10^{-4} (3.36 \times 10^6 \Delta T)^{1/4}$$

It is this equation into which we insert the values of ΔT within the liquid (except for $\Delta T < 0.003^\circ\text{C}$ as indicated above) to obtain the values of H_v (which is \bar{h} times the vertical cylindrical area) to use in our calculations. For convenience \bar{h} was plotted against ΔT within the liquid in Figure C-1. It varies from 0.0559 W/cm²°C for a ΔT of 10 to 0.0039 for a ΔT of 10^{-4}°C .

2. Heat Transfer from Bath to Calorimeter

We wish to calculate the thermal conductance in the design of Figure 11 of the text across the helium gas from the external bath to the metal bottle holder in the calorimeter. There are three parallel paths of heat flow we can visualize. One is the shortest direct line across the space. Another is across the space above the holder and down through the inner glass wall. The third is across the space to the internal heat sink and up through the thermocouple system to the bottle holder. Call the conductances corresponding to these three pathways H_1 , H_2 , and H_3 .



**FIG. C-1 : THE CONVECTIVE HEAT TRANSFER COEFFICIENT FOR THE
SAMPLE SOLUTION AS A FUNCTION OF THE TEMPERATURE
DROP IN THE SOLUTION.**

The surface for H_1 is 5 cm high so

$$A_1 = \pi \times 3.45 \times 5 = 54.2 \text{ cm}^2$$

Thus for two thicknesses of 1.5 mm glass with a k of $0.011 \text{ W/cm}^\circ\text{C}$ and 4-mm of helium with a k of $1.5 \times 10^{-3} \text{ W/cm}^\circ\text{C}$

$$H_1 = \frac{54.2}{\frac{0.3}{0.011} + \frac{0.4}{0.0015}} = 0.184 \text{ W}^\circ\text{C}$$

In a similar fashion we calculate the conductance for the area above the holder, but the heat must then be conducted down through the glass to reach the holder so the total conductance is much less and

$$H_2 = 0.007 \text{ W}^\circ\text{C}$$

For the copper heat sink there is more area involved.

$$A_3 = \pi \times 3.45 \times 7 + \frac{\pi}{2} 3.45^2 = 94.6 \text{ cm}^2$$

Then using the other figures the same as for H_1 , we get for the conductance to the sink $0.322 \text{ W}^\circ\text{C}$. Elsewhere (see Appendix C-3) it is shown that the conductance from the sink to the bottle is $0.0725 \text{ W}^\circ\text{C}$. This combines reciprocally with the above figure to give for H_3 a value of $0.059 \text{ W}^\circ\text{C}$. H_1 , H_2 , and H_3 then add directly to give a total thermal conductance across the helium gas of $H_{He} = 0.250 \text{ W}^\circ\text{C}$.

If instead of considering the gap filled with helium we did the exact same calculation for mercury using the k of liquid mercury, $0.091 \text{ W/cm}^\circ\text{C}$, we get $H_{Hg} = 1.790 \text{ W}^\circ\text{C}$.

3. Thermopile Construction and Thermal Conductance

It was decided to use a copper-constantan thermopile constructed by a plating technique in the design. Solid state thermo-elements such as bismuth telluride might possibly be substituted with very little change in the design, but not having full information on their characteristics, we considered metal couples in these design calculations.

To construct the plated couples, we started with two solid plastic cylinders, 7 mm diameter and 19 mm long. On each we close wound 125 turns of 36 gage enamelled constantan wire. (36 gage wire is 0.127 mm diameter; with enamel this increases to 0.141 mm; thus $125 \times 0.141 = 17.6 \text{ mm}$. If this number of turns runs too close to the ends, a few less turns could be used.) To produce a thermopile, the enamel is removed from the outer surface with fine emery cloth; the cylinder is held horizontally and dipped

exactly half way into a plating solution and plated with copper. Now when the cylinders are held between two surfaces with the plated-unplated junctions at the surfaces there are 125 pairs of copper-constantan junctions in series on each cylinder to measure the difference between the surfaces. Construction of similar couples are discussed by W. E. Evans. *

For our use we must consider the heat conduction of the thermopile as well as the thermoelectric power. Since copper has almost twenty times the thermal conductivity of constantan, the thickness of the plating is important. Removing the enamel as described would probably bare about a fourth of the circumference of the wire. Platings one fifth of the wire diameter in thickness have been successfully used in construction of such thermopiles. Thus we estimate the cross-sectional area of the copper as $0.100 \times 0.025 = 0.0025 \text{ mm}^2$. That of the constantan is $\frac{\pi}{4} \times 0.127^2 = 0.0127 \text{ mm}^2$. Since the thermal conductivity of copper is $3.86 \text{ W/cm}^\circ\text{C}$ and that of constantan is $0.218 \text{ W/cm}^\circ\text{C}$ and the length of each section on the 7 mm cylinder is 11.2 mm, the conductance of the unplated arm is:

$$H_{\text{cons}} = \frac{kA}{\ell} = \frac{0.218 \times 0.0127}{11.2} \times \frac{1}{10} = 2.47 \times 10^{-5} \text{ W}^\circ\text{C}$$

$$\text{that of the plating } H_{\text{Cu}} = \frac{3.86 \times 0.0025}{11.2} \times \frac{1}{10} = 8.62 \times 10^{-5} \text{ W}^\circ\text{C}$$

$$\text{that of the plated arm is } H_{\text{plated}} = (2.47 + 8.62) \times 10^{-5} = 11.09 \times 10^{-5} \text{ W}^\circ\text{C}$$

$$\text{and that of each pair is } H_{\text{(pair)}} = (11.09 + 2.47) \times 10^{-5} = 1.356 \times 10^{-4} \text{ W}^\circ\text{C}$$

4. Heat Flow Paths and System Sensitivity

We will now calculate thermal conductance values for all the heat flow paths in the apparatus. Through all the couples we have

$$H_{\text{couples}} = 250 \times 1.356 \times 10^{-4} = 0.0339 \text{ W}^\circ\text{C}$$

The larger this last figure is in relation to the other flows, the more sensitive and more stable the design will be. The couples are mounted in a disk of plastic 30 mm in diameter and 7 mm thickness. Use $k = 0.00144 \text{ W/cm}^\circ\text{C}$ as an average value for hard plastics. $A = \frac{\pi}{4} 30^2 = 707 \text{ mm}^2$

$$H_{\text{plastic}} = \frac{0.00144 \times 707}{7} \times \frac{1}{10} = 0.0145 \text{ W}^\circ\text{C}$$

* W. E. Evans in "Biochemical Microcalorimetry", H. D. Brown, Ed. (Academic Press, NY, 1969), p 266.

There will be conduction down the glass of the inside wall of the dewar. This will be an annular area with 32.5 mm average diameter, 1.5 mm width and will be 7 mm long. $A = \pi \times 32.5 \times 1.5 = 153.2 \text{ mm}^2$

Use 0.011 W/cm°C for the k of glass.

$$H_{\text{glass}} = \frac{0.011 \times 153.2}{7} \times \frac{1}{10} = 0.0241 \text{ W/}^\circ\text{C}$$

If the sample chamber and heat sink do not fit tightly in the dewar this figure would be smaller. However, then it might be variable and contribute to unsteadiness. The total flow from sample to sink is: $0.0339 + 0.0145 + 0.0241 = 0.0725 \text{ W/}^\circ\text{C}$.

Next we calculate a conductance value for the heat flow up from the sample. Through the glass-straight part for which $A = 153.2 \text{ mm}^2$, $\ell = 46 \text{ mm}$

$$\frac{kA}{\ell} = \frac{0.011 \times 153.2}{46} \times \frac{1}{10} = 0.00366 \text{ W/}^\circ\text{C}$$

Through the glass-curved part

$$A_{\text{av}} = \frac{32.5\pi + 43.5\pi}{2} \times 1.5 = 179 \text{ mm}^2$$

$$\ell = \frac{5.5\pi}{2} = 8.64 \text{ mm}$$

$$\frac{kA}{\ell} = \frac{0.011 \times 179}{8.64} \times \frac{1}{10} = 0.0228 \text{ W/}^\circ\text{C}$$

Since these are in series their reciprocals add as follows:

$$H_{\text{glass}} = \frac{1}{\frac{1}{0.00366} + \frac{1}{0.0228}} = 0.00315 \text{ W/}^\circ\text{C}$$

The plug will be insulating material; assume $k = 0.00038 \text{ W/}^\circ\text{C}$

$$\frac{kA}{\ell} = \frac{0.00038 \times 707}{44} \times \frac{1}{10} = 0.00061 \text{ W/}^\circ\text{C}$$

So the conductance value for the total heat flow upward is 0.000376 W/°C

As a preliminary indication of sensitivity we can calculate what it would be if the heat sink were not isolated but communicated with the bath. Then using $40 \mu\text{V}/^\circ\text{C}$ as the average sensitivity of a copper-constantan thermocouple,

$$\frac{(0.0725 + 0.0038) \text{ W}/^\circ\text{C}}{(40 \times 10^{-6} \times 250) \text{ V}/^\circ\text{C}} = 7.63 \frac{\mu\text{W}}{\mu\text{V}}$$

Comparing this with the $18.5 \frac{\mu\text{W}}{\mu\text{V}}$ value for the calorimeter used to obtain the data in this report, we see that there is plenty of leeway for loss of sensitivity due to isolation of the heat sink. Calculation of the response of the actual design is considerably more complicated. It is presented in Appendix D.

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APPENDIX D

Appendix D

Estimated Response of Calorimeter Design Concept

In the preceding section it was implied that unlike the design calorimeter, the Mound Calorimeter had a fixed calibration constant. That this can only be applied directly to the observed temperature difference under equilibrium conditions such as may be attained with an electric calibration was seen earlier; when ΔT is varying, a correction for changing heat content of the sample and container must be applied to calculate more closely the true heat production rate. With the design calorimeter it is necessary to consider both changing heat content of the calorimeter cell and contents and the slowly changing temperature of the isolated heat sink used as a temperature reference, so even with a constant power input we can have no fixed calibration constant. The impact on the measurements of using an isolated heat sink as a temperature reference must be assessed.

In order to calculate the response we proceed as follows:

Let T_s = temperature of the sample

P = power generated in sample

T_c = temperature of heat sink

C_c = heat capacity of sink

H_c = thermal conductance, sample to sink

T_a = constant temperature of bath surrounding calorimeter

H_a = thermal conductance, sample to surrounding bath

P will be given as a function of time, probably in tabular form, and T_s and T_c are to be calculated with $T_s - T_c$ being the desired result.

$$P = C_s \frac{dT_s}{dt} + H_a(T_s - T_a) + H_c(T_s - T_c) \quad (1)$$

$$C_c \frac{dT_c}{dt} = H_c(T_s - T_c) \quad (2)$$

With P constant or expressed analytically, these could probably be solved analytically. But with P in tabular form, a numerical form of solution is necessary. If the table is not in equal intervals of time, dt, it should be placed in this form. Then using the notation:

$$\Delta T_s = T_s - T_a \quad (3)$$

$$\Delta T_c = T_s - T_c \quad (4)$$

We have for each interval, dt,

$$P_{av} = \frac{P_t + P_{t+dt}}{2} \quad (5)$$

$$\Delta T_{s_{av}} = \Delta T_{s_t} + \frac{dT_s}{2} \quad (6)$$

$$\Delta T_{c_{av}} = \Delta T_{c_t} + \frac{dT_s - dT_c}{2} \quad (7)$$

Then rewriting (2) in this interval form

$$\frac{dT_c}{dt} = \frac{H_c}{C_c} \Delta T_{c_{av}} \quad (8)$$

Combining (8) and (7) we have

$$dT_c = \frac{H_c dt}{C_c} \Delta T_{c_t} + \frac{H_c dt}{C_c} \frac{dT_s}{2} - \frac{H_c dt}{C_c} \frac{dT_c}{2} \quad (9)$$

$$dT_c = \frac{\frac{H_c dt}{C_c} \Delta T_{c_t} + \frac{H_c dt}{2C_c} dT_s}{\frac{H_c dt}{2C_c} + 1} \quad (10)$$

$$dT_c = B \Delta T_{c_t} + 1/2 B dT_s \quad (11)$$

$$\text{where } B = \frac{\frac{H_c dt}{C_c}}{\frac{H_c dt}{2C_c} + 1} \quad (12)$$

Rewriting (1) in interval form we have:

$$P_{av} = C_s \frac{dT_s}{dt} + H_a \Delta T_{s_{av}} + H_c \Delta T_{c_{av}} \quad (13)$$

Combining (13) with (6) and (7) we have:

$$P_{av} - H_a \Delta T_{s_t} - H_c \Delta T_{c_t} = \left(\frac{C_s}{dt} + \frac{H_a}{2} + \frac{H_c}{2} \right) dT_s - \frac{H_c}{2} dT_c \quad (14)$$

Then putting (11) into (14)

$$P_{av} - H_a \Delta T_{s_t} - H_c \Delta T_{c_t} = \left(\frac{C_s}{dt} + \frac{H_a}{2} + \frac{H_c}{2} \right) dT_s - \frac{H_c}{2} B \Delta T_{c_t} - \frac{H_c B}{4} dT_s \quad (15)$$

Rearranging

$$dT_s = \frac{P_{av} - H_a \Delta T_{s_t} + H_c (B/2 - 1) \Delta T_{c_t}}{C_s/dt + H_a/2 + H_c/2 - H_c B/4} \quad (16)$$

In using this expression, at the start of an interval we know ΔT_{s_t} and ΔT_{c_t} .

For the interval we calculate P_{av} from (5), dT_s from (16) and dT_c from (11) and for the end of the interval we have:

$$\Delta T_{s_t+dt} = \Delta T_{s_t} + dT_s \quad (17)$$

$$\text{and } \Delta T_{c_t+dt} = \Delta T_{c_t} + dT_s - dT_c \quad (18)$$

These then serve as the values for the start of the next interval and so on. For these calculations we must also calculate the heat capacity of the sample and its immediate surroundings as follows:

Sample: 24 g water

Bottle (20 g of glass): $20 \times 0.186 = 3.72$ g water equivalent

Copper cup: $V = 30 \pi \times 1 \times 49 \times 1/4 \pi 31^2 \times 1$

$$= 5,373 \text{ mm}^3$$

$$W = 5.37 \times 8.92 = 47.9 \text{ g Cu}$$

$$47.9 \times 0.092 = 4.41 \text{ g water equivalent}$$

Glass in contact with copper cup:

$$V = 32.5 \times \pi \times 1.5 \times 50$$

$$= 7658 \text{ mm}^3$$

$$W = 7.658 \times 2.23 = 17.08 \text{ g glass}$$

$$17.08 \times 0.186$$

$$3.18 \text{ g water equivalent}$$

Sum:

$$35.3 \text{ g water equivalent}$$

$$\text{Therefore: } C_s = 35.3 \text{ Cal/}^\circ\text{C} = 147.7 \text{ J/}^\circ\text{C}$$

Similarly we calculate C_c for the copper heat sink:

$$V = 1/12\pi 303 + 1/4\pi 30^2 \times 60 = 49,480 \text{ mm}^3$$

$$W = 49.5 \times 8.92 = 441.4 \text{ g Cu}$$

and therefore

$$C_c = 441.4 \times 0.092 = 40.61 \text{ Cal/}^\circ\text{C} = 169 \text{ J/}^\circ\text{C}$$

From Appendix C-4 we know

$$H_c = 0.0725 \text{ W/}^\circ\text{C} \text{ and } H_a = 0.00376 \text{ W/}^\circ\text{C}$$

Now using these data and if we let $dt = 900 \text{ sec}$ we have from (16) and (11) and repeating (17) and (18) and with P_{av} in watts:

$$dT_s = 5.08 P_{av} - 0.0191\Delta T_{s_t} - 0.309\Delta T_{c_t} \quad (19)$$

$$dT_c = 0.322\Delta T_{c_t} + 0.161 dT_s \quad (20)$$

$$\Delta T_{t+dt} = \Delta T_{s_t} + dT_s \quad (17)$$

$$\Delta T_{c_t+dt} = \Delta T_{c_t} + dT_s + dT_c \quad (18)$$

Finally for the 250 copper-constantan couples we have, using a thermoelectric power of $40 \mu\text{V/}^\circ\text{C}$

$$V = 250 \times 40 \Delta T_c = 10^4 \Delta T_c \mu\text{V.}$$

We have used this approach to calculate the response on the design calorimeter (design as presented in Figure 11 of the text) for comparison with the observed response of the Mound Calorimeter to the bacteria *Salmonella typhimurium* (4 cells/ml). The results are shown on Figure D-1. We have also calculated the response for a constant power input. This is also shown on Figure D-1. When compared with the observed responses of the Mound Calorimeter, we see that with the design calorimeter the response to a constant power input does not maintain an equilibrium value indefinitely as it does with the Mound, but the signal decreases very slowly from the peak value as the isolated copper heat sink used as a temperature reference warms up. However the calculated response curves, when compared with the observed curves, show that the use of this reference temperature block does not impair the usefulness of the apparatus in detecting bacterial heat outputs in the calorimeter during the early stages of the runs. Thus this design element which has other desirable design attributes for the total system can be used for our application without concern.

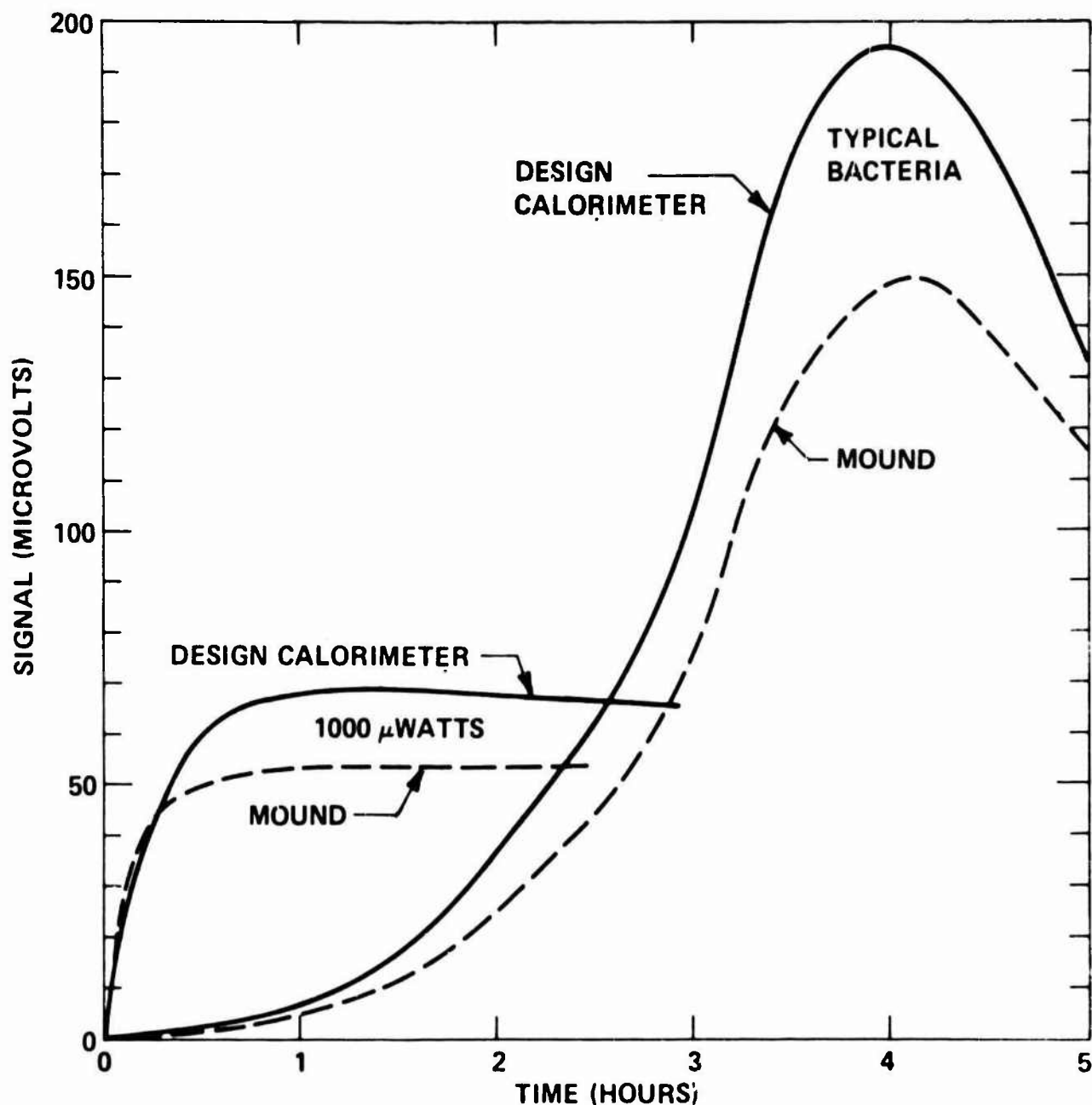


FIG. D-1 : CALCULATED OUTPUTS FOR THE CALORIMETER DESIGN OF FIG. 11 COMPARED WITH OBSERVED OUTPUTS FOR THE MOUND CALORIMETER (BASED ON HEAT PRODUCTION OF SALMONELLA TYPH.)